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PROBLEMS OF DEVELOPMENT[1]

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WILLIAM THOMPSON SEDGWICK, in whose memory the lectureship was established which I have the honor to occupy on this occasion, was one of the founders and incorporators of the Marine Biological Laboratory, one of its first board of seven trustees, in which capacity he continued for twelve years, and one of the first lecturers at this laboratory. It is most appropriate, therefore, that these facts should be recalled on this occasion and in this place.

One of the outstanding characteristics of Professor Sedgwick was the breadth of his interests and sympathies, and these qualities have become characteristic of this laboratory. Its founders, chief among whom were Hyatt, Minot and Sedgwick, were men of broad interests and generous purposes; and its first director, Professor Whitman, dedicated it to be "a national center of research in every department of biology." In an age of specialization we must all be specialists in research, but this is no excuse for narrowness of sympathy or for intolerance. Bigotry is as hateful in science as anywhere else. Problems and methods vary from decade to decade; fashions in research are as much in evidence as in dress. Perhaps it is but natural that those clothed in the newest styles should look with amused superiority

[1] William Thompson Sedgwick Memorial Lecture, delivered at the Marine Biological Laboratory, Woods Hole, Mass., July 27, 1928.

upon those in older garments. But it would be a sad day for this laboratory if it should ever lose its spirit of catholicity, of cordial sympathy with good work in any field. Almost every biological problem can be approached from many sides, and in its final solution many methods are needed. The success of the Marine Biological Laboratory in the past has been the result of the cordial cooperation of persons working in different fields, and this spirit it owes in the main to the breadth of view of its founders.

I wish on this occasion to discuss some of the problems of development which have occupied the attention of probably more investigators at this laboratory than have any others. If this discussion deals largely with work done at this laboratory it is because time is lacking for a more complete presentation, and if it is concerned chiefly with the visible structures and functions rather than with the more fundamental physics and chemistry of development, it is not due to lack of interest in these other aspects of this subject, but rather to the necessity of specialization. Furthermore, I would suggest that morphological studies are necessary preliminaries to the biophysics and biochemistry of development; and for the rest I can only hope for such tolerance from those who have left cells and nuclei for ions and electrons as I trust may be accorded to them by a coming generation.

Problems of genesis are central in all science and philosophy. Of all methods of biological analysis, the genetic method has yielded the most important results, especially when combined with experiment; and of all biological problems ontogeny is probably the most inclusive since it concerns almost every phase of inquiry in every field of biology. Furthermore, the development of an egg is an epitome of all development, whether of races or species, of structures or functions, of animals or man, of mind or society. It is not to be wondered at that development has ever been the central problem of biology, nor that marine laboratories, with their unrivaled

opportunities for the experimental study of the development of free eggs and embryos, have been centers of research in this field.

Development is progressive differentiation, coordinated in time and place, and leading to specific ends. There are innumerable problems of development, indeed almost every problem associated with living things finds illustration and illumination in developing organisms. It is as true to-day as it was just one hundred years ago, when von Baer wrote it, that "Die Entwicklungsgeschichte ist der wahre Lichtträger für Untersuchungen über organische Körper." But the chief problems of development may be grouped around the three phrases of the definition just given; namely, (1) When and how do progressive differentiations arise? (2) How are coordination, orientation and regulation brought about? (3) How can one explain the teleological character of development where the end seems to be in view from the beginning? Only the first of these general problems has received adequate treatment hitherto; an important beginning has been made on problems of coordination and regulation; but with respect to the teleological character of development we are still almost completely in the dark, and many biologists regard this as no problem at all; that is, they explain it by explaining it away, or else they deny that it is a problem for scientific investigation and turn it over to philosophers or theologians.

I. The Progressive Differentiations of Development

All cells, even the simplest, are differentiated, and recent genetical studies have been showing how much more complex the germ cells are than any microscopist could ever have demonstrated or dreamed. But the complexities discovered by geneticists are located in the chromosomes, and so far as known the chromosomes do not undergo progressive differentiation in the course of ontogeny. The differentiations of development can not

be attributed to a differential distribution of chromosomes to the cells of the embryo as Roux and Weismann assumed, for under typical conditions every cell has the same chromosomal constitution. The chromosomes, then, are carried on from generation to generation, usually without any significant change in their constitution except such as results from new combinations that are made in maturation and fertilization, and progressive differentiation is limited to portions of the cell outside the chromosomes. And yet there is demonstrable evidence that the unchanging genes in the chromosomes are most important factors in the progressive changes in the cytoplasm, though of course they can not be the only factors of differentiation, for according to the principle of scientific determinism it is impossible that identical causes should produce different effects.

Interrelations of Nucleus and Cytoplasm. The earliest steps in differentiation consist in the formation in the cell body of specific substances as a result of the interaction of nucleus and cytoplasm, and the general method of this interaction can be readily seen in the development of the egg. During intervals between successive cell divisions the nucleus grows by absorbing soluble, dialyzable substances from the cell body, whereas when the nuclear membrane is dissolved in mitosis a cloud of nuclear material, which could not diffuse through the nuclear membrane, is set free into the cell body. Thus the nucleus receives relatively simple food materials and gives out elaborated products. In the earliest stages of oogenesis and spermatogenesis the nucleus at its maximum size nearly fills the entire cell; almost all of the material in the cell body at this stage is absorbed by the nucleus in its growth, thus showing that at this stage most of the cell contents are dialyzable; but as development proceeds and differentiation products appear in the cell body the quantity of cytoplasmic material which can not be absorbed by the nucleus in its growth increases, while the relative volume of the nucleus decreases.

Finally, in the mature ovum, although the nucleus is large, it is still only a fraction of the volume of the cell body; and in later stages of differentiation the ratio of nucleus to cytoplasm continues to grow less until it may be only one hundredth part or less of the volume of the entire cell (Conklin, 1912). Minot (1908) interpreted this decrease in the nucleus-plasma ratio as the cause of senescence, but it in turn is caused by the increase of non-dialyzable differentiation products in the cell body. In general, the relative size of the nucleus is inversely proportional to the volume of differentiation products in the cell, and conversely the degree of differentiation of a cell can usually be judged by the relative size of its nucleus.[2] The earliest stages of the germ cells have relatively the largest nuclei because most of the substances in the cell body can enter the nucleus by dialysis, but, as a result of the liberation of nuclear materials into the cell body and their combination there with cytoplasmic materials, non-dialyzable differentiation products accumulate outside of the nucleus. Either because different kinds of materials are taken into or liberated from the nucleus at different stages, or more probably because the same kinds of substances are set free from the nucleus and combine at different stages with different materials in the cell body, there is a progressive formation of differentiation products in the cell. It is a matter of common observation that while the division of chromosomes is non-differential, that of the cell body is often differential, and the same chromosomes and genes acting upon different kinds of cytoplasm, through the same kinds of liberated nuclear materials, would be expected to produce different results. Here is a visible mechanism by which differentiation products are formed in eggs and cleavage cells.

[2] Nerve cells in general have large nuclei, which indicates that they contain a large quantity of fluid in addition to their fibrillae; muscle cells have relatively small nuclei, indicating that their fibrillae fill most of these cells; many gland cells have small, shrunken nuclei when filled with secretion products, but much larger nuclei after these products have been discharged.

But differential cell division is the result not only of the formation of different substances in different cells but also of definite movements and segregations of the cytoplasm, of definite orientations of mitotic figures and cleavage planes, and ultimately of a definite polarity and symmetry of the cell body. These cytoplasmic orientations are not the immediate results of chromosomal activity, though they may be the more remote results of such activity at an earlier period, but in any event it is logically impossible to place all the differential factors of development in non-differentiating chromosomes and genes.

Hypotheses of Nuclear Control of the Cell. Several hypotheses have been advanced to explain the "nuclear control of the cell" or, more exactly, the relation of inheritance factors in the chromosomes to the differentiations of ontogeny. It must be said that at present such hypotheses are wholly tentative but they serve a useful purpose if they help to define the problem and to suggest methods of solving it. Mental pictures or models are a necessary part of the furniture of the scientific mind. One of the first of the hypotheses for explaining the nuclear control of the cell was that of "intracellular pangenesis" proposed by deVries in 1889. He suggested that units which he called "pangenes" and which he regarded as enzymatic in nature escape from the nucleus into the cell body where they form all the living parts of the cell. Most of the nuclear pangenes were supposed to be inactive at any particular stage, but they might become active by age or by surrounding conditions. The pangenes which escape from the nucleus must be transported to different parts of the cell and segregated at the right places; this transportation and localization he supposed was brought about by the streaming of the protoplasm.

This hypothesis of deVries has served as a model for almost every hypothesis that has been advanced since; even the name "gene" is a modified form of "pangene";

but in a few respects his conception has undergone important modification as a result of further research. De Vries held that "every inherited character has its particular kind of pangene." This conception has suffered the same fate as has befallen all more recent views that have regarded inheritance factors as representatives of developed characters. Genes are not the germs of such characters any more than hydrogen and oxygen are the germs of water; on the other hand, they are specific, intrinsic factors which, acting in conjunction with other factors, both intrinsic and extrinsic, give rise to developed characters. Furthermore, it is not now supposed that genes escape as such into the cytoplasm, or that the entire cytoplasm is composed of them; but it is known that there is an escape of a relatively large quantity of nuclear material into the cell body at every mitotic division and that, as a result of this, new substances are formed and localized in the cytoplasm. This, in brief, is the mechanism which is generally supposed to link genes with cytoplasmic differentiations.

Morgan, in his Sedgwick Lecture of two years ago (1926), reviewed several of the more recent hypotheses of nuclear control of development by means of enzymes, hypotheses which have been put forward by some ten or more different geneticists or physiologists. After a keen criticism of some of the weaknesses and defects of these hypotheses, and after showing that we know too little regarding the nature of genes and their action in development to justify at present any theories upon the subject, he concludes, "While we may not be warranted in speaking of genes as enzymes, the genes may be protein bodies, one of whose activities is to provide enzymes, which, being set free, act in each cell and take part in catalytic reactions in the cytoplasm." This conclusion seems to coincide in broad outlines, though not in all details, with the views that have been generally held since the hypothesis of deVries.

One of the most recent and extensive theories of the relation of genetics to development is the "Physiolo-

gische Theorie der Vererbung" of Goldschmidt (1927). Indeed, it represents for modern genetics and developmental mechanics what Weismann's "Germplasm" did for the earlier work on heredity in relation to ontogeny and phylogeny. Like Weismann's theory, it is in part more detailed and complete than the facts at our disposal at present would justify, if one is inclined to be severely critical; in part it is founded on the author's conclusion that genes differ not only qualitatively but also quantitatively, and that such phenomena as sex, dominance, multiple allelomorphism, and mutation are determined by such quantitative differences in genes—a view which is not generally accepted by geneticists. Goldschmidt's theory is presented with a wealth of illustrative examples which adds materially to its value, and in its main features it furnishes a working model of the possible or probable relations of genes to the physiology of development.

In his lecture in this place last summer (1927), Lillie dealt with the relation of genes to the physiology of development in a manner which I think is more searching than in any other similar contribution to this subject. Further reference will be made to this lecture later, but it may be said here that after an illuminating analysis of the physiology of development and a review of the attempts to explain these phenomena by genetics, he concludes that between the genes and the ontogenetic process there is a great gulf that has never yet been bridged.

This very brief outline of prevalent views regarding the relation of genes to ontogeny may be sufficient, in view of previous lectures in this place and on this subject, to indicate that this is one of the major problems of development. We have no such knowledge regarding the physiological activity of genes as Morgan and his associates have so convincingly and so brilliantly furnished regarding what Weismann called "the architecture of the germ plasm."

Differentiations of the Germ Cells. I turn at once to some of the cellular aspects of development and the

microscopical evidences of progressive differentiation. Both male and female germ cells have undergone considerable development before their union in fertilization. This is plainly the case with the spermatozoon, which is one of the most highly differentiated of all cells. Most of these differentiations appear in the spermatid after the last maturation division and they represent the end of development, as it were the end of the life cycle, of these cells; whereas the end of development of the egg-cell is not reached until the end of the life cycle of the individual that develops from the egg. In short, the spermatid corresponds in stage of development to the ootid; but the mature spermatozoon, which has undergone extensive differentiation after the last maturation division, does not correspond to the egg at the close of its maturation divisions but rather to the individual that develops from a parthenogenetic egg. The spermatozoon is an organism with phyletic, generic, even specific characteristics, with peculiarities of movement as well as of structure, and the old zoologists were nearly right when they named it the "sperm animal."

The differentiations of a spermatozoon are confined to a single cell, whereas those of a developing egg are usually distributed among multitudes of cells. But while the distinction between unicellular and multicellular organisms is one of great significance, it does not constitute any fundamental contrariety between the two. In his stimulating lecture on "The Inadequacy of the Cell Theory of Development," Professor Whitman (1893) showed that essentially similar structures may be composed of one cell or many cells, and Lillie (1902) found that the egg of the annelid Chaetopterus might undergo a certain amount of differentiation in the absence of cleavage, giving rise to ciliated forms which in some cases resembled normal trochophores. I have recently found that when cleavage is suppressed but nuclear divisions continue in the fertilized eggs of the ascidian, *Styela partita,* the typical localization of the

cytoplasms peculiar to the ectoderm, mesoderm and endoderm, the typical peculiarities of the nuclei in these areas, and even some of the histological differentiations of the tadpole stage and of its metamorphosis, may sometimes appear. However, the localization of these plasmas takes place normally at the time of the first cleavage and is provided for before that event; it is therefore not dependent on cleavage. All traces of later differentiations of these unsegmented eggs are abnormal in form and location, and to a large extent this is true also in Chaetopterus, as described by Lillie.

Undoubtedly cell-division plays an important part in ontogenetic differentiations; it facilitates interchange between nucleus and cytoplasm, it stimulates intracellular movements of orientation and localization, it leads to the more complete isolation of different substances; but all of these things may take place in the absence of cell-division, as we see in many highly differentiated protozoa, spermatozoa and tissue cells. In fact, the fundamental features of differentiation are the same in unicellular and in multicellular forms. Many processes in the development of a spermatozoon parallel in miniature those of the immensely larger and longer development of the egg. Among these parallel processes one notes (1) the importance of cytoplasmic movements in the two cases leading to the localization of different substances and structures in the spermatozoon or in the egg and embryo; (2) the similar significance of phase membranes and cell membranes in the isolation of these different substances and in physiological processes in general; (3) the development of polarity and symmetry in both spermatozoon and egg or embryo; (4) no modification in the number or genetic constitution of the chromosomes and genes is caused by these cytoplasmic differentiations of the spermatozoon or multicellular organism; (5) the elongation of the spermatozoon as well as of the embryo occurs because of the growth in length of supporting structures, such as the axial fiber or the notochord.

In conclusion, the essential processes of ontogenetic differentiation may be seen in miniature in the differentiation of a single cell, but the amazing differences in the results of development in the male and female germ cells with apparently so slight an initial difference in the chromosomal and cytoplasmic constitution of the spermatid and ootid, or the enormous differences between certain tissue cells with apparently identical chromosomal constitution—these things deserve much more serious study than they have received hitherto.

Promorphology of the Egg. As contrasted with the differentiations of a mature spermatozoon, the visible differentiations of an egg at the time of fertilization are relatively few, but they are of great importance in ontogeny. Probably all eggs show polar differentiation before fertilization, usually by the greater concentration of cytoplasm at one pole and of yolk at the other. In most cases this is not due, as was once supposed, to mere differences in the specific weights of these substances, for they occupy no constant position in relation to the direction of gravity. In Crepidula and other gasteropods the relative positions of yolk and cytoplasm may be reversed by means of centrifugal force (Conklin, 1910, 1917), but these substances tend to return to their original positions when this force is discontinued. In these cases, and probably in many others, the polarity of the egg resides in the hyaloplasm, or ground substance, which forms a layer at the periphery and a framework throughout the interior of the egg. This hyaloplasm is most concentrated at the animal pole, and conversely the yolk is crowded to the opposite pole. By centrifugal force the heavier yolk may be forced into the hyaloplasm at the animal pole, but as soon as this pressure is removed it tends to resume its original position. Lillie (1906, 1909) found essentially similar conditions in the eggs of the annelid Chaetopterus; in one of the most careful studies that has ever been made on Echinoderm eggs, Hörstadius (1928) concludes that the polarity consists in a gradation

of concentrations of the plasma, or in a stratification of dissimilar kinds of plasmas in the animal-vegetative axis.

There are relatively few cases in which a bilateral arrangement of egg substances can be seen before fertilization. A few eggs, such as those of insects and cephalopods, have all the axes of the future animal marked out in the egg while it is still in the ovary. Other eggs are bilateral in internal structure just before or after fertilization, as is the case in the pigeon, frog, amphioxus and ascidians. In still other cases, bilaterality or asymmetry does not become visible until much later stages of development.

In addition to polarity and symmetry, some eggs have areas of differentiated materials that in the course of development go into particular organs; consequently all such materials were once called "organ-forming substances." However, experiments, especially with centrifugal force, by means of which substances differing in specific weight may be dislocated from their normal positions, have generally shown that after the most extreme dislocations or even complete elimination of these materials normal development may still occur if the nucleus and transparent hyaloplasm are left intact. This proves conclusively that such materials as oil, yolk and pigments are not "organ-forming substances," but it does not prove that every part of the hyaloplasm of the egg is undifferentiated or that there are no organ-forming regions in the hyaloplasm. The very fact that these visible materials become localized in particular areas indicates that these areas must differ in some respect from others. In short, these inclusions serve as "indicators" of some fundamental difference in various areas of the hyaloplasm.

The method of segregation and localization of visibly different substances in the polar or bilateral axes or in the organ-forming areas can be followed especially well in the living eggs of the ascidian *Styela partita*. When the germinal vesicle breaks down in the prophase of the

first maturation division there is liberated into the cell body near the animal pole a very large amount of transparent nuclear sap, linin and oxychromatin; the surface of the egg is at this time covered by a layer of hyaloplasm in which are imbedded yellow spherules of a lipoid substance and many mitochondrial granules; the remainder of the egg contains gray yolk spherules imbedded in a hyaloplasmic matrix. At the moment when the spermatozoon enters the egg near the vegetative pole, the superficial layer containing the yellow spherules flows rapidly to the point of entrance where it forms a yellow cap on the surface, and at the same time most of the transparent cytoplasm collects in a zone immediately above the yellow cap,[3] leaving most of the yolk in the animal half of the egg. The sperm nucleus and aster then move up to the equator on the posterior side and the yellow material follows them, and as the cleavage amphiaster is formed this yellow material spreads out on the surface in the form of a crescent immediately over the amphiaster. At the same time the zone of clear cytoplasm moves to the equator where it forms a clear crescent just above the yellow crescent, while most of the yolk is rotated into the anterior half of the egg. During the first cleavage this rotation of the clear cytoplasm and yolk continues until, at its close, they occupy their definitive positions, the clear cytoplasm in the upper half of the egg and the yolk in the lower half. At the same time a light-gray crescent appears on the anterior side of the egg, opposite the yellow crescent, which corresponds in position and in potency to the "gray crescent" of the frog's egg.

These segregations and localizations are the results of movements within the egg which are apparently induced by the entrance of the spermatozoon, its movement through the egg and the formation of the cleavage amphiaster. But on the other hand there is good evidence that the region of entrance of the sperm, its path within

[3] Meves (1913) does not find such a zone in Phallusia but it is very evident in Styela, as is shown in my photomicrographs of living eggs (1905, Figs. 1 and 2).

the egg, the location of the cleavage spindle posterior to the middle of the egg and at right angles to its chief axis, are determined by the organization of the cytoplasm. This is true also of the movements of nuclei and spindles during later cleavages, since these movements are preceded and caused by movements in the cytoplasm. Similarly in the maturation, fertilization and cleavage of Crepidula, movements of the cytoplasm, although they may be induced by nuclei or asters, are directed by the organization of the cytoplasm itself.

Sooner or later localization of different materials in specific areas takes place in all eggs or embryos, but the pattern and time of localization and the histological character of these substances differ in various phyla of animals. Sometimes such localizations are clearly marked even before cleavage; in other cases they are not visibly present at much later stages when experiments reveal the fact that they are actually existent; furthermore there are differences in the completeness of segregation, some species showing a sharp separation of different substances, others only a greater or less aggregation of particular substances in definite areas.

The ascidian egg represents an extreme case of early and relatively complete segregation of at least four different substances: namely, the clear cytoplasm which gives rise to ectoderm and may therefore be called ectoplasm; the deeply staining cytoplasm, containing the yellow spherules and mitochondria which goes into the muscles and mesenchyme and may be called mesoplasm; the yolk-laden plasm that becomes endoderm and may be called endoplasm; and the material of the light-gray crescent which gives origin to the notochord and neural plate and may therefore be called chorda-neuroplasm. The segregation and localization of all these substances is accomplished before the close of the first cleavage. Similar localizations occur in the frog's egg, and other patterns of localization are found in many other animals. These localizations of different cytoplasmic materials

mark out the relative position and proportions of future organs; they constitute, as Brachet (1927) has said, "the material, dynamic and proportional substratum of the adult animal."

Nevertheless, most of the visible inclusions in these different areas are not "organ-forming." In Styela some eggs contain little or no yellow pigment and yet they develop in a perfectly normal manner. On the other hand, eggs in which the yellow pigment is displaced from its normal position by centrifugal force usually develop abnormally. Recently I have found that this is due to the dislocation of the cytoplasmic area in which the pigment lies and not to the dislocation of the pigment itself. If the centrifugal force is relatively little the yellow spherules and the heavier yolk may sometimes be displaced from their usual positions without seriously interfering with normal development. But by very strong centrifuging different areas of the cytoplasm itself—different chemically and physically as shown by their staining reactions, viscosity and microscopical texture—may be dislocated, and in such cases development is never normal; even the cleavage cells are atypical in form and position as well as in contents and each of these different kinds of cytoplasm, if it develops at all, gives rise to its own specific part or organ of the larva wherever it may be located. In this way the most bizarre monsters may be formed, with their different organs out of all proper relation to one another, recalling the teratoid tumors of pathology or the "membra disjecta" of myth and fable.

On the other hand, whenever individual blastomeres of the ascidian egg are destroyed the embryo that develops from the cells that are left always lacks those parts and organs which would have come from the cells which were destroyed. Thus there is both positive and negative proof that, apart from such inclusions as oil, pigment and yolk, there are organ-forming areas and substances in the cytoplasm of the eggs of ascidians—positive in that such cytoplasmic materials may be displaced from their nor-

mal positions and relations to other materials in the egg and yet give rise to the characteristic structures and tissues which they would have produced if they had remained in their usual positions, negative in that if the cells containing these materials are destroyed the resulting embryo lacks the parts which would have come from these cells and substances if they had continued to develop.

However, the ascidian egg represents an extreme case of mosaic development, and in this connection it is interesting to note that its rate of differentiation is unusually rapid. In *Styela partita,* the fully formed tadpole stage is reached in twelve hours after fertilization; in *Molgula manhattensis,* a tadpole with brain, sense organs and spinal cord, with muscles, notochord and alimentary canal—in fact a perfect little vertebrate—develops in six hours after fertilization; whereas in a frog or toad a similar stage is not usually reached in less than two weeks. It is highly probable that this early and rapid differentiation is associated causally with the extremely mosaic character of development in ascidian eggs.

The great variability in the degree of differentiation of unsegmented eggs of different species applies equally well to their cleavage stages. In the nineties of the last century Driesch (1891 *et seq.*) maintained as a result of his experiments on echinoderm eggs that "by segmentation perfectly homogeneous parts are formed capable of any fate." At the same time Wilson (1892 *et seq.*) and other workers at this laboratory were finding that cleavage in several different animal phyla was a definite, stereotyped process of segregation and isolation of differentiated areas and materials of the cytoplasm, and their experiments on isolated blastomeres proved that in these eggs development is, in the language of Roux, "a mosaic work" of cells having specialized materials and limited potencies. Hence arose the distinction between "mosaic" and "regulative" eggs, "determinate" and "indeterminate" cleavage.

These differences among various animals were for a long time a sore puzzle to embryologists. The literature of experimental embryology is still filled with conflicting accounts based on these differences. One investigator, despairing of finding a unifying principle, lightly suggested that perhaps "every egg is a law unto itself." At last order is coming out of this confusion, the explanation of these discrepancies being found chiefly in differences in the time and rate of differentiation in various animals. Sooner or later differentiations of materials and cells, and restrictions of potencies appear in all animal eggs and embryos, and these perplexing discrepancies are due mainly to the time at which such differentiations and restrictions appear.

These early differentiations of polarity, symmetry and pattern of localization have long been known as the "promorphology" of the egg, a name which indicates that they are causally related to the morphology of the embryo. Unlike the differentiations of the spermatozoon, which are largely lost when it enters the egg, these early differentiations of the ovum are the foundations of embryonic differentiations. They determine the polarity, symmetry, type of cleavage, pattern of localization and general plan of development. Not until after these earlier and more fundamental differentiations have occurred does the effect of the genes brought into the egg by the spermatozoon begin to be felt. Consequently, while the share of the egg and the spermatozoon is approximately equal in later stages of development, as is shown by reciprocal crosses, their share in the early stages is not equal, since the promorphology of the egg determines the type of early development and consequently the general plan and pattern of the embryo. And this promorphology has developed either as the direct result of the activity of the genes in the ovarian egg or through stimuli or "inductions" received from egg membranes, the position of the egg in the ovary or its relation to other maternal tissues, which in turn are

conditioned by maternal genes—that is, it is the result of "maternal inheritance." In short, ontogeny begins before fertilization, and therefore the share of the egg in development is greater than that of the spermatozoon. But in deference to the general equality of the sexes I should add that the share of the maternal grandfather is probably as great as that of the grandmother in determining the promorphology of the egg.

It would be interesting to know to what extent this promorphology of the egg is the result of the activity of genes within the egg-cell and how much is due to the immediate environment, for, of course, both intrinsic and extrinsic factors are involved in the development of a spermatozoon or ovum as well as in that of all later stages. Morgan (1927, p. 181) says that it is not known whether the bilateral structure of the squid's egg is determined in the first instance by the shape of the follicle which surrounds it, or by the protoplasm of the egg itself. But since this is a constant character it is as certainly inherited as is any other character and its cause must therefore be traced back to the germ plasm of the female, even though there may be many links in the chain between the genes and the developed character. Since the immediate environment in the case of the germ cells is the body of the parent, which in turn is the product of both heredity and environment, it is particularly difficult to distinguish between these two classes of factors in the differentiations of the egg or sperm. Only where it is possible to cross-breed forms having different types of germ cells and then to study the germ cells of the progeny can one decide with certainty what is to be ascribed to heredity and what to environment. Unfortunately, crosses between forms differing so widely as this are usually impossible, but one such case is so universally realized that it is usually disregarded in this connection; namely, the cross between males and females of the same species, but having germ cells as different as spermatozoa and ova. In this case it is known with certainty that

sex, and consequently the type of sex-cells formed, is a Mendelian character. Whatever influences environment may exert in the development of subordinate features of germ cells, it is certain that initially genes are concerned in the determination of sex and consequently of the two different types of germ cells.

There is also some additional evidence that the polarity, symmetry and pattern of the egg are conditioned by genes, although they may also be influenced by environment. One interesting case in which it has been possible to investigate the origin of the promorphology of the egg by observation, experiment and breeding is found in certain species of gasteropods where one variety has dextral and the other sinistral asymmetry. Many years ago Crampton (1894) and also Kofoid (1894) found that some of the early cleavages of the eggs of sinistral snails were the mirrored images of those of the dextral variety, and I found that this inverse symmetry could be traced back to the first cleavage, and very probably to the unsegmented egg (Conklin, 1903); I suggested that if the polarity of an egg which already had spiral, bilateral or asymmetrical structure could be reversed without changing the relative positions of the substances in the cross axes of the egg there would result inverse symmetry of every stage from the first cleavage to the mature form. Many attempts were made to reverse the polarity of eggs by subjecting them to centrifugal force, but while the yolk could be driven to the animal pole and the cytoplasm and nucleus to the vegetative pole and even the polar bodies could be caused to form at a distance from the animal pole, nevertheless the original polarity was reestablished whenever this was possible. Many dislocations of organs occurred but only one case of inversion or partial inversion of symmetry in a snail at the time of hatching (Conklin, 1910). Within the past year Hämmerling (1927) has studied this problem in the case of amphibian eggs which were inverted before cleavage. He finds that the polarity of the egg can be reversed, but this does not

usually, if ever, lead to *situs inversus* in the tadpole, though it often causes partial inversion or dislocation of organs. It is certain that the inverse symmetry of sinistral as contrasted with dextral gasteropods can be traced back to the unsegmented egg, but it is not certain that a dextral type of egg can be changed into a sinistral one, or *vice versa,* by centrifugal force.

Several attempts have been made to analyze the causes of inverse symmetry by cross-breeding dextral and sinistral snails, but the results have been so varied that it has been impossible to find any general interpretation of them. Lang (1904) found that the occasional cases of inverse symmetry in Helex are not inherited, but Boycott and Diver (1923) show that there is some evidence in Limnaea that they are inherited in an irregular manner. Sturtevant (1923) suggested that these results might be due to "maternal inheritance," namely, that the dextral or sinistral condition is already present in the egg before fertilization and that it has therefore been determined by maternal genes. Consequently, in cross-breeding, paternal genes would affect the symmetry only in the F_2 generation. If this interpretation is correct, it means not only that the polarity and asymmetry of snails are inherited but that both develop during oogenesis and are present as such in the eggs before they are fertilized. If this view is the true one the most fundamental features in the promorphology of the egg, namely, polarity and symmetry, are inherited as are other characters, but since such inheritance appears in the egg before fertilization it is "maternal inheritance" or "preinheritance" (Conklin, 1916).

Physiology of Development. In answer, then, to our first inquiry, when and how do progressive differentiations arise, it may be said that we have the outlines of a fairly satisfactory mechanistic process, although there are certain serious gaps in the picture. Differentiations occur at every stage in ontogeny from the formation of the egg in the ovary to the end of development. Genes

as well as the cellular substances surrounding them are factors in this process at every stage. By some interaction between genes and other nuclear or cytoplasmic materials specific chemical substances are formed in the cell body, and by means of intracellular movements these substances are localized in definite regions of the egg and are afterwards isolated by phase membranes or cell walls. Similar processes of segregation and isolation continue throughout the cleavage and early embryology, the earlier specializations inducing differentiations in unspecialized cells and areas, and leading to unequal growths, invaginations and organ formations. Lastly, individual cells in the various organ-forming areas undergo their final histological differentiations by the same processes of formation of specific substances and their localization.

At every stage in this process the entire organism—that is, genes, nucleus, cytoplasm and specific formative substances—reacts to stimuli whether intra-organic or extra-organic, and each particular stage is the necessary precursor of each succeeding one. The egg is like a complicated machine that is wound up to go in a particular manner, but unlike any machine of human invention it is so constructed that it transforms itself at every critical stage into another machine with its own peculiar modes of action; and so the living, moving, transforming mechanism runs on from stage to stage.

Development is a series of responses to stimuli, each comparable to the specific responses of tissue cells. A muscle cell when stimulated contracts, a gland cell secretes, an egg cell develops; only in the case of the egg the system itself changes from stage to stage so that the responses are not merely repetitive, as in the case of tissue cells, but progressive. At many of the earlier stages of ontogeny, and in some cases even in later stages, the developing organism may respond in different ways, depending upon the particular conditions or stimuli that act upon it. Lillie (1927) maintains that at each such

stage there are two and only two possible methods of response; that is, at every critical point of development two alternative paths are open, one of which is taken and the other left; but once a particular path has been taken the same alternatives do not occur again. These branching paths occur much more frequently in regulative eggs than in mosaic ones, where they may be almost entirely lacking, and in all cases they become fewer as development progresses and in many cases disappear altogether. Lillie has proposed the useful terms "open" and "closed" for the stages and types of development in which alternatives are offered or in which there are no alternatives. Thus the ectoderm of the gastrula of amphibia is an open system in that it may give rise to ordinary ectoderm or to neural plate, the latter depending upon some stimulus or "induction" from the cells in the dorsal wall of the archenteron, as has been so beautifully demonstrated by Spemann and his associates. At a later stage ectoderm may remain as ordinary epithelium of the skin or may, if stimulated by contact with the optic cup, produce the lens of the eye. Similar conditions are found in open systems at earlier stages of development, as, for example, in isolated blastomeres of echinoderm eggs. On the other hand, there are species in which the system is a closed one even at very early stages, as in the case of isolated blastomeres of the eggs of annelids, mollusks and ascidians, where every blastomere gives rise only to those parts which it would have produced if it had remained in its normal relation to other blastomeres; between these two extremes many intermediate conditions are found.

In conclusion, it must be admitted that this explanation of the processes of differentiation is incomplete. We observe a series of mechanisms but we do not know what the precise nature of these mechanisms is, and least of all do we know how they have arisen. A greater problem than the mechanism of development is the development of this mechanism.

II. Problems of Orientation, Coordination and Regulation

Concerning our second major problem, namely, how are coordination, orientation and regulation brought about, recent researches have added much to our knowledge without furnishing any final solution. What are the methods and means of coordination in development? What controls the time and place of action of the genes and other factors? What fixes the rate of differentiation in various parts and in different species? More accessible than any of these questions and no less important—what determines the orientation of movements upon which the orderly localization of all substances and processes depends? We know very little about any of these problems. Even the time and rate of cell-division varies greatly in different cells and areas of the same embryo, and presumably this is due to differences in their physical and chemical constitution, though there is often no direct evidence of such differences.

Orientation. The orientation of intracellular movements by which different substances are segregated and localized and the mitotic spindles turned into particular positions so that cleavage takes a characteristic form for each species is one of the most important processes in development. In some eggs, such as those of some polyclads, nematodes, mollusks and ascidians, these movements can be watched under the microscope and studied experimentally. They are stimulated by the escape of nuclear material into the cell body at the time of mitosis. They stop in the absence of oxygen and are disorientated by various physical and chemical changes in the environment such as cold, hypotonic or hypertonic solutions, etc. In Crepidula these intracellular movements are apparently caused by the contractility of the hyaloplasm and they seem to be directed by chemotaxis, for at the close of every cleavage the centrosomes and asters move to the free surface of the cell nearest to the animal pole, but in the absence of oxygen at the free surface this movement

does not take place. It is probable that the viscosity and chemical constitution of the hyaloplasm, which as we have seen differs in different areas, is one of the directing factors; but there is a lack of critical evidence upon this point.

The orientations of these movements in eggs and blastomeres, the active changes in the shapes of cells, as, for example, in the gastrulation of ascidians where the ectodermal epithelium becomes flattened and the endodermal column, thus causing invagination; the continued growth of cells in one axis, as in the notochord—all of these and many other similar phenomena give evidence of being mechanistic phenomena capable of a physiological explanation; and yet we do not know what causes even the circulation of protoplasm in a plant cell or an ameba, and much less do we know what causes and directs the movements of chromosomes, centrosomes, amphiasters and formative materials in eggs and embryos.

Coordination and Integration. How are all the processes of development coordinated and integrated? Thirty-five years ago Whitman in a lecture at this laboratory emphasized the fact that the organism is one and the same individual at every stage of its development, whether it consists of one cell or of many. Although the organism may be subdivided into many parts or cells, under normal conditions it is still a single whole. This may signify that some sort of living bond connects all the parts; such a bond has been found by several investigators in an ectoplasmic layer that surrounds all the cells in the cleavage stages, and by intercellular bridges of protoplasm in later stages, but whether such bonds are universally present or in themselves are sufficient to explain the unity of the organism are still open questions. In relatively late stages of ontogeny living bonds are furnished by means of nerve connections, and it is interesting and perhaps significant that in plants where nerve connections are lacking, intercellular bridges are apparently more common than in animals. Finally, it is known

that in later stages of development there are chemical means of integration through hormones, and it is possible that these also occur in early stages and that they are the most important means of bringing about integration and coordination. It seems probable that the "organizers," which Spemann and his associates have found such important factors in amphibian development, are of this nature.

Regulation. In certain cases of regulative development adult organisms, embryos or egg cells may be divided into two or more parts and each of these may then become a complete organism of smaller size, or the reverse of this may occur and two eggs be caused to fuse and give rise to a single embryo of double size. In either of these cases integration into complete individuals takes place either by a rearrangement of substances in the egg and of cells in the embryo or by new differentiations of unspecialized cells. In general, regulation is more perfect in the egg and embryo than in the adult, because differentiation is less complete in the former than in the latter, but there is at least one remarkable exception to this rule. The eggs and embryos of ascidians are almost wholly without regulative capacity, but the adults have extraordinary powers of regulation and regeneration. Recently Spek (1927) has offered an interesting explanation of this anomaly. He finds that all regeneration in Clavellina is due to the activities of certain small ameboid cells containing drops of a protein or lipoid substance, and not to any activity of differentiated cells. These "drop cells" are unspecialized and totipotent. They are derived, at least in part, from the walls of the intestine, and in potency they are equivalent to undifferentiated cells.

Causes of regulation and reintegration are among the most perplexing problems of development. A perfectly determinate, non-regulative type of development could be conceived of in a strictly mechanistic, materialistic way; to be sure, the mechanism would need to be wonderfully

complex, but still if it always went in one way and to one end there would be good reason to maintain that it is a pure mechanism. But regulative development—and all development is more or less regulative—in which displaced substances resume their typical positions, isolated blastomeres give rise to whole embryos "as if the pattern of the whole were in every part," and in which the predestined fate of substances and cells is completely altered in order, if I may so say, to carry out the typical plan—how is it possible to explain such regulation and restoration in a mechanistic manner? In this respect the development of mosaic eggs, which are generally regarded as a later and more highly organized type, is easier to understand than that of the more primitive regulative eggs.

Several of the keenest investigators who have ever dealt with this problem have felt constrained to postulate some non-mechanistic factor. Chief among these is Driesch, who has contributed so much to our knowledge of regulation. Despairing of finding a causal explanation, he resorts to a form of vitalism in which entelechies, which are non-mechanistic, direct the physiological processes. His first and chief evidence of vitalism is that the egg and embryo can not be regarded as a machine because it is impossible to conceive of any machine that can be fragmented in the three dimensions of space and the fragments still be capable of forming a complete machine, as was thought to be true of the egg. But this may mean no more than that the living machine is more complex than any Driesch has in mind. We know that the organism consists of machines within machines. The inner machine in every cell is the nucleus, usually containing two sets of chromosomes and genes, any one set of which is capable of giving rise to an entire organism if it is not prevented by the outer machine consisting of cytoplasm and the products of differentiation. Thus it may be possible to conceive of a machine that can be fragmented in the three dimensions of space and yet be capable of

giving rise to a whole machine. But it is true that at present it is not possible to fill in all the details of such a model.

III. The Teleology of Development

We can not at present offer a purely mechanistic explanation of regulation, or, for that matter, of orientation, or of any other process in the whole course of development. And this brings us to our last great problem, namely, how can one explain the apparent teleology of development where the end seems to be in view from the beginning? Development is indeed the most perfect example of teleology in all nature. Consider the teleological character of every stage in this process, the genes and their marshaling in time and place; chromosomes and their synapsis and reduction upon which the whole of Mendelism depends; the adaptive structures of ova and spermatozoa; consider the adaptations to particular ends in mitosis and cell-division; the formation, segregation, localization of specialized substances; the coordination and induction of parts; the orientation and regulation of all developmental processes. From beginning to end it appears that development is moving toward a goal. Substances appear at the right time and place for future needs; adaptations appear in cleavage, as Lillie (1899) showed long ago; organs develop in anticipation of future needs, eyes and sense organs while the embryo is still protected from the special stimuli for which they are fitted. In short, the needs of the future organism are anticipated at every step. Spemann, whose experimental analysis of the development of the amphibian egg is the admiration of the scientific world, said in his rectorial address at the University of Freiburg (1923), "Nature acts in development as an artist making a picture or model; indeed, as every organizer does who handles materials whether living or not living. . . . This differs from Weismann's purely mechanistic theories and resembles our own activities," that is, it is appar-

ently purposive and in the same sense in which our activities are purposive.

I shall not weary you with a recital of the many historic attempts to solve this problem, but shall merely recall that they fall into two categories, the vitalistic and the mechanistic. The former assumes a perfecting principle, indwelling soul, *vis formativa, vis essentialis,* entelechy or *élan vital,* as the guiding and directing factor. This factor is acknowledgedly beyond the reach of science and the scientific method. Such an assumption virtually declares the mystery insoluble and therefore removes the strongest stimulus to further research. We must therefore as scientific workers refuse the "Ruhekisse" of vitalism and seek our solution of the problem in mechanism. But at the same time we should not close our eyes to facts even if we can not deal with them mechanistically. To refuse to recognize the teleological aspect of development does not eliminate it; we can not explain it by merely explaining it away, for the problem remains and future generations will deal with it if we can not.

Two major attempts have been made to explain adaptations in a mechanistic way, namely, Lamarckism and Darwinism, and the teleological character of development is only one very striking case of organic adaptation in which the organism is fitted to future conditions as well as to present ones. Both the Lamarckian and the Darwinian explanations are based upon the cyclical nature of development from egg to adult to egg to adult in practically endless succession. According to the former, fitness is the direct result of the incorporation of the experiences or acquirements of previous cycles in succeeding ones. If the experiences of one cycle could be impressed upon the next by the inheritance of acquired characters there would be a basis for the doctrine of "precocious segregation" proposed by Lankester or for the "law of acceleration" of Hyatt, according to which adult characteristics are carried back to earlier and

earlier stages in ontogeny. But there is no satisfactory evidence for the inheritance of such acquirements.

On the other hand, the Darwinian explanation of fitness, namely, multifarious variation and the elimination of the unfit, applies to every stage of development as well as to adult organisms. For every adult that is eliminated by natural selection scores of embryos and larvae and hundreds of eggs are found unfit, and as fitness here applies to future needs as well as to present ones, it would be possible to explain even the teleological character of development in a mechanistic manner, if it always ran a constant course, as in mosaic eggs. But how is it possible to explain regulative development where the plan is changed to meet new conditions and where the "organizer" in the embryo "acts as an artist making a picture or model." Most of all, how is it possible by mechanistic science to explain the purposive activity of the artist, for whether there be anything in the developing organism resembling purpose there is no doubt that there is something which we call "purpose" in human life and, if mechanism is universal, sooner or later science must deal with this greatest of problems. We have been finding that the fundamental properties of all living things are similar and that science and the scientific method can be applied to man as well as to other organisms. The development of intelligence, of will, of consciousness in man is a natural process if anything in the world is natural, and by "natural" we mean what Bishop Butler and Darwin meant, namely, "that which is stated, fixed, settled"—in short that which is lawful and causal, or deterministic and mechanistic.

Comparative and developmental psychology are revealing the steps by which intellect, will and consciousness develop; we are learning that in psychical as well as in physical development the method of trial and error and finally trial and success is probably universal. This is the method of elimination or selection of reactions rather

than of organisms and while we call it "artificial" or "purposive" when accompanied by human intelligence it is none the less natural. In the development of intelligence and purpose all kinds of actions, ideas and ideals are tried; those that fail to give satisfaction are eliminated and those that succeed are retained. Purpose in man and teleology in organisms may be fundamentally alike, in which case the "organizer" in development would "resemble our own activities" as Spemann has suggested, and yet both of these be causal phenomena and therefore subjects for scientific investigation. Here is the greatest problem of development, and one which so far from discouraging research should greatly stimulate it.

The chain of cause and effect is endless and every cause discovered leads to inquiries as to the cause of this cause. We trace differentiations to inductions and these to earlier formations and localizations of formative materials and these to the promorphology of the egg, and all of these to genes—only to be met by the eternal question of the cause of this last link. We find mechanisms of differentiation only to inquire as to the causes of these mechanisms. We find orientations, regulations and teleology only to be mystified by the immensity and complexity of these problems of development. But this is the method and these are the limitations of science, for nature is infinite and our science touches only the hem of her garment. But so far from discouraging research it should stimulate us to know that we are working in a field which has no limits and that our explorations will never end, will never lead to an *Ultima Thule* because there is no such place.

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EXPERIMENTAL STUDIES ON THE DURATION OF LIFE

XII. INFLUENCE OF TEMPERATURE DURING THE LARVAL PERIOD AND ADULT LIFE ON THE DURATION OF THE LIFE OF THE IMAGO OF *DROSOPHILA MELANOGASTER*[1]

W. W. ALPATOV[2] AND RAYMOND PEARL

I

THE origin of the present investigation was closely connected with a study of the influence of temperature on physical characteristics of *Drosophila* conducted by one of the authors (W. W. A.) in the Institute for Biological Research during the winter of 1927–1928. The flies developed under different temperatures showed such pronounced differences in the body size as well as in the coloration, that it was decided to test their longevity. In order to have a larger material for discussion it was thought desirable to test the duration of life of "cold" and "warm" flies in three incubators running at different temperatures. As a result of this expansion of the problem the double character of the investigation arises. The first part of it concerns the problem of the influence of the different temperatures on the duration of life of flies genetically and phaenotypically identical; the second is devoted to the question of the duration of imaginal life in connection with different conditions of larval life, *i.e.*, development under different temperatures.

Up to the present time both of these problems have been only occasionally and insufficiently attacked by other investigators, and have not been worked out in the exact form adopted by Pearl and his coworkers from the experimental as well as the statistical points of view.

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Very few investigations can be mentioned in connection with these problems. The most important are those of Loeb (100), Loeb and Northrop (14) and Northrop (17). In Loeb's first paper on the influence of temperature on the larvae of sea urchins the temperature coefficient obtained differs considerably from the usual temperature coefficients for different biological processes. This diversity compelled Loeb to emphasize what he regarded as the peculiar nature of the phenomenon of senescence, in comparison with other biological processes. His second paper on *Drosophila* gave quite different results in regard to the magnitude of the temperature characteristic of the duration of life, showing that in this case the process of senescence apparently falls within the limits of other biological processes influenced by temperature. At the present time we know that the temperature coefficients of life processes vary considerably within the temperature scale. Loeb's experiments on the mortality of larvae of the sea urchin subjected to different temperatures were run at the right marginal side of the temperature scale, and the data can not be compared with data on temperature coefficients of other biological processes obtained in the middle of the scale. So far as we know, Loeb himself never tried to reconcile his findings of 1908 with his later observations on *Drosophila.*

Loeb and Northrop's paper (16) contains the first results on the relation of the duration of the three stages of life of *Drosophila* at different temperatures. These authors state that "as far as our present experiments go the ratio of the duration of life of the insect to the duration of the larval stage is approximately constant for all temperatures and that the same is true for the ratio of the larval to the pupal stage." Our own observations upon the time of development in the eggs showed that there is an extremely wide range of variation depending on the degree of development in which the egg is at the moment of oviposition. At a temperature 28° C. the range of

eclosion is between a few hours and 24 hours, the largest number of eclosions falling in the interval between 18 and 24 hours after oviposition. This means that the determination of the beginning of the larval period, even if the period of oviposition is reduced to two hours, is far from being accurate enough. In Loeb and Northrop's experiments (16) the procedure is even less precise. The authors write: "Aseptic flies of both sexes were put in flasks and allowed to remain 15 hours at room temperature, during which time a number of eggs were laid. . . . The larvae hatch in a few hours after the eggs are laid, and at the time the flies were removed from the flask most of the larvae had already hatched. The duration of life of the larvae was reckoned from the time the eggs were placed in the incubator to the time the pupae were formed." Bonnier (95) used a much more accurate method for the determination of the beginning of the prepupal period of the development. He placed fertilized flies on the food and allowed them to oviposit only two hours. The result was that Bonnier succeeded in showing, with very good evidence, that "the shortening of the time of development at 30° C. as compared with the time at 25° C. is much more pronounced for the pupal than for the prepupal stage." It can be seen that this conclusion is just opposite to Loeb and Northrop's statement based, as we believe, on a technique insufficiently precise. A detailed comparison of Loeb and Northrop's data with our own will be made later on. Northrop's (17) paper has also a certain relation to our problem. He attempted to prolong the larval life by insufficient feeding and to find if such a prolongation influences the duration of the imaginal life. His method of obtaining larvae was even less precise than that described in Loeb and Northrop's paper. This consequently produced a considerable heterogeneity in respect to the age distribution of the population in his experimental flasks. The question of the correlation between the length of larval

and imaginal life must be regarded as still an open one.

We can not refer to all the papers devoted to the influence of temperature on the rate of development of other insects than *Drosophila*. They are very numerous but only a few can be mentioned as fulfilling the requirements of an exact experimental work. To this category belong the papers of Peairs (102), Titschack (105), Krogh (99), Janisch (97), and Bliss (94).

In connection with our second problem—the size of the body and the duration of life—only one paper may be mentioned. This is the article of Titschack (106), cited from Janisch (98). This author found that the larger moths had also a longer duration of life.

II

The flies used in our experiments belong to the wild culture of *Drosophila melanogaster* known as Line 107 (Pearl and Parker, 32). On December 1, 1927, the parents of experimental flies were taken from sixteen stock bottles, each starting with five pairs of flies. One hundred bottles were populated with five pairs each and divided into two parts (50 and 50); one series was placed in an incubator running at 18° C., the other in an incubator at 28° C. The parents were kept on the food for six days. From the fifty bottles kept at 28° C. in 47 bottles flies had emerged on December 9. On December 10 the 28° C. bottles gave material representing the "warm" flies. The flies were collected on December 10. On the day before all the flies which had then emerged were shaken from the 28° C. bottles. Therefore the age of the flies at the moment of beginning the duration of life experiment was between 0 and 24 hours. The flies were placed in one-ounce bottles, 25 males and 25 females in each. The bottles were divided into three groups and placed in incubators running respectively at 18°, 25°, and 28° C.

The emergence of flies in the bottles kept at 18° C. was naturally more delayed. Emerged flies were observed

in 15 bottles on December 19, and in 38 on December 20. The "cold" flies which emerged on December 21 furnished the material for incubators running at 18° and 25° C. Those which emerged on December 22 were put in the incubator running at 28° C. In calculating the average duration of life we did not count this 0–24-hour period, calculating the age of our flies from the moment of putting the flies in the ounce bottles.

In this work we used for the temperatures of 28° and 25° ordinary electric incubators, and for the temperature of 18° C. a Hearson low temperature incubator, with an ice-box and electrical heating. We were obliged to use 25° C. as an intermediate temperature because of the difficulty in running an electric incubator at 23° C., this temperature being too close to the room temperature of the laboratory. At that time we did not have a second Hearson incubator, which would have allowed us to have the desired temperature of 23° C. Besides having accurate chemical thermometers the incubators were provided with thermographs. The temperature records were taken twice a day, at 9 A. M. and 5 P. M. We can not say that the temperature in the above mentioned incubators can be kept extremely constant. Calculating biometrical constants for morning temperatures of the first 100 days of the experiment we get the data shown in Table 1.

TABLE 1

CONSTANTS FOR TEMPERATURE VARIATION IN THE THREE INCUBATORS

Constant	18°	25°	28°
Mean	18.04° ± .04	25.13° ± .03	28.00° ± .04
Standard deviation	0.65°	0.52°	0.62°
Coefficient of variation	3.58 ± .17	2.05 ± .10	2.22 ± .11
Limits	16.1° – 20.0°	23.1° – 27.3°	25.8° – 30.3°

It must be noted that in the incubator running at 18° C. the temperature fell three times to 8°–10° C., due to faulty operation of the regulatory apparatus. According to the

thermographic records this undercooling took place only for a few hours and apparently did not influence the vitality of the experimental animals. However, these three exceptional cases are not included in the data which gave us the material for the calculation of Table 1. It can be seen that although the variation of temperature is rather broad, the extreme deviations occurred so rarely that the averages are very close to the desired temperatures. The greater part of the data is concentrated, in fact, within $\pm 1°$ limits from the average temperature. This fact is made apparent biometrically by the very low coefficients of variation.

III

Turning attention first to the influence of temperature on the time of development of *Drosophila,* it is to be noted that this phenomenon exhibits considerable time variation. We observed two points: first, the moment of putting the parental flies on the food; and, second, the average time of appearance of the emerged flies in the bottles. The same data were recorded concerning the development of flies which were bred for the purpose of a biometrical study. As can be seen from Table 2, the

TABLE 2

AVERAGE TIME (IN DAYS) FROM THE BEGINNING OF THE CULTURE UNTIL THE EMERGENCE OF THE FLIES

Material	18°	25°	28°	30°
Our material prepared for duration of life experiment	19.00 ± .08	—	8.06 ± .02	—
Our material for measurements	16.25 ± .15	9.58 ± .04	8.55 ± .12	—
Bonnier's data	—	9.6	—	7.6

total duration of development at 28° C. is approximately half as long as that at 18° C.

The physical characteristics of flies developed at different temperatures have been measured on individuals originating from a specially organized experiment. The history of the animals of that experiment is as follows. On October 20, 1927, 20 pairs (♂ and ♀) were taken from the mass culture of the Wild Line 107, and put into 20 half-pint bottles with 100 cc synthetic food. The most fertile bottles (according to the first and second days of emergence) gave origin for brother and sister matings (♂ and ♀) in which virgin females were used.

From one of the most fertile of these brother × sister bottles, flies were taken and placed (5♂ and 5 ♀) in three

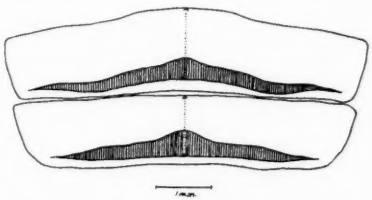

FIG. 1. Points defining the measurements of the length of the fifth and sixth tergum.

bottles each and placed in the incubator with corresponding temperature. The first generation of flies developed under different conditions was measured, 50 males and 50 females being taken for each temperature. The following measurements were chosen:

(1) Width of the head (including the compound eyes).
(2) Length of the fifth tergum (assuming that the first tergum is morphologically a double segment) (Fig. 1).
(3) Length of the sixth tergum (Fig. 1).
(4) Length of the femur (middle pair of legs) (Fig. 2).
(5) Length of the tibia (middle pair of legs) (Fig. 2).
(6) Distance between the anterior and posterior crossveins (Fig. 3).
(7) Length of the posterior crossvein (Fig. 3).
(8) Length of the wing (Fig. 3).
(9) Width of the wing (Fig. 3).

The measurements were made with the aid of a Spencer screw ocular micrometer; the first seven measurements

were made with a Leitz No. 3 objective, and the eighth and ninth with a Reichert No. 1 objective, in both cases on a Spencer microscope. The material was preserved in 70 per cent. alcohol and before measuring it was mounted under cover glasses in glycerin.

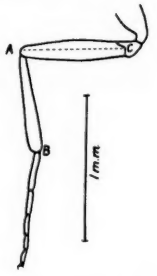

FIG. 2. Points defining the measurements of the length of the tibia (A–B) and the femur (A–C) of the middle pair of legs.

The accompanying Tables 3 and 4 show the differences which exist between flies belonging to the different experimental series. In the central column of these tables R denotes the ratio of the difference between corresponding means to its probable error. The number of char-

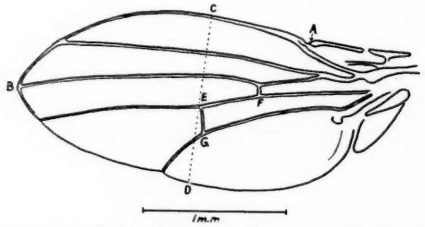

FIG. 3. Measurements of the wing. AB, length of the wing. EF, distance between the anterior and posterior crossveins. CD, width of the wing. EG, length of the posterior crossvein.

acters measured on males was reduced. Characters 2 and 3 were omitted because of the difficulties in straightening the tergums on the slide. The coefficients of variation in these two characters in the females are extra-

TABLE 3

BIOMETRICAL CONSTANTS FOR FEMALES OF DROSOPHILA REARED AT 18° AND 28° C.

Characters	♀ development at 18° C.			Differences in means	♀ development at 28° C.		
	Mean	Standard deviation	C. of V.		Mean	Standard deviation	C. of V.
1	0.9054 ± .0021*	0.0216	2.39 ± .16	0.0627 ± .0030 R = 20.9	0.8427 ± .0022	0.0228	2.71 ± .18
2	0.2834 ± .0021	0.0219	7.73 ± .52	0.0003 ± .0027 R = 0.1	0.2831 ± .0017	0.0173	6.11 ± .41
3	0.2913 ± .0020	0.0207	7.11 ± .48	0.0064 ± .0028 R = 2.4	0.2846 ± .0020	0.0204	7.52 ± .51
4	0.7070 ± .0014	0.0146	2.07 ± .14	0.0402 ± .0021 R = 19.1	0.6668 ± .0015	0.0156	2.34 ± .16
5	0.7246 ± .0016	0.0172	2.37 ± .16	0.0433 ± .0021 R = 20.6	0.6813 ± .0013	0.0135	1.98 ± .13
6	0.4954 ± .0017	0.0180	3.63 ± .24	0.0614 ± .0024 R = 25.6	0.4340 ± .0016	0.0167	3.84 ± .26
7	0.1990 ± .0008	0.0083	4.17 ± .28	0.0178 ± .0012 R = 14.8	0.1812 ± .0009	0.0092	5.08 ± .34
8	1.8832 ± .0033	0.0343	1.82 ± .12	0.1837 ± .0045 R = 40.8	1.6995 ± .0030	0.0312	1.84 ± .12
9	1.0690 ± .0017	0.0187	1.75 ± .12	0.1007 ± .0024 R = 42.0	0.9683 ± .0017	0.0169	1.75 ± .12

* All measurements are in millimeters.

TABLE 4

BIOMETRICAL CONSTANTS FOR MALES OF DROSOPHILA REARED AT 18° AND 28° C.

Characters	♂ development at 18° C.			Differences in means	♂ development at 28° C.		
	Mean	Standard deviation	C. of V.		Mean	Standard deviation	C. of V.
1	0.8290 ± .0021*	0.0218	2.63 ± .18	0.0248 ± .0030 R = 8.3	0.8042 ± .0021	0.0220	2.74 ± .18
4	0.6766 ± .0010	0.0105	1.55 ± .10	0.0472 ± .0017 R = 27.8	0.6294 ± .0014	0.0151	2.40 ± .16
5	0.6937 ± .0013	0.0135	1.95 ± .13	0.0477 ± .0021 R = 22.7	0.6460 ± .0016	0.0165	2.55 ± .17
8	1.7310 ± .0018	0.0190	1.10 ± .07	0.2324 ± .0038 R = 61.2	1.4986 ± .0034	0.0352	2.35 ± .16
9	1.0124 ± .0014	0.0151	1.49 ± .10	0.1324 ± .0024 R = 55.2	0.8800 ± .0019	0.0201	2.28 ± .15

* All measurements are in millimeters.

ordinarily high. This is due without doubt, *not* primarily to the organic variation of that particular characteristic, but to the distortions arising during the dissection and mounting of the object on the slide.

It is evident from Tables 3 and 4 that the *flies, females as well as males, developed at the low temperature are significantly larger* in all characters other than 2 and 3 than are the warm temperature ones. Besides these differences in the dimensions of the body another very interesting peculiarity of the cold temperature flies must be mentioned. This is in the development of the black spots on the tergum and at the base of the wings (see Fig. 4). It is to be noted that the seventh tergal spot,

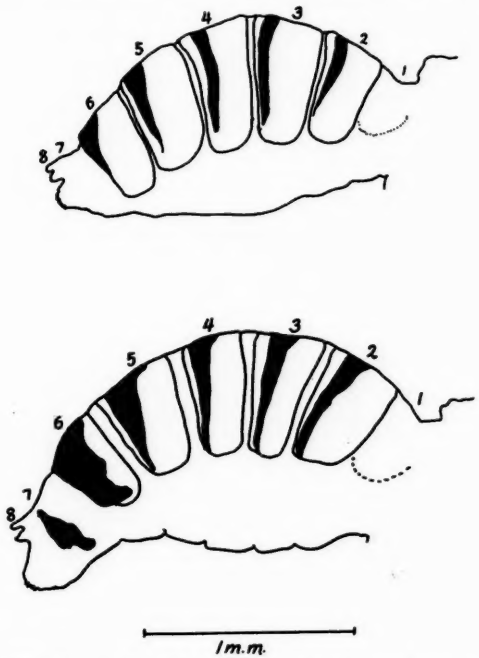

FIG. 4. Side view on the abdomen of two female specimens of *Drosophila melanogaster*. Above is a specimen from the 28° culture, below one from the 18° culture. The drawings were made with a camera lucida to the same degree of magnification.

TABLE 5

SURVIVORSHIP DISTRIBUTIONS OF FLIES UNDER DIFFERENT CONDITIONS AS TO THE TEMPERATURE OF DEVELOPMENT AND IMAGINAL LIFE

Temperature of development / Temperature of imaginal life	18°/18°		18°/25°		18°/28°		28°/18°		28°/25°		28°/28°	
Days	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
0– 4	1,000*	1,000*	1,000*	1,000*	1,000*	1,000*	1,000*	1,000*	1,000*	1,000*	1,000*	1,000*
5– 9	996	986	986	986	992	966	976	970	978	978	982	968
10– 14	992	982	982	984	986	964	974	966	966	956	966	962
15– 19	980	974	954	958	878	928	962	958	880	909	884	890
20– 24	942	968	774	872	522	710	918	940	502	833	488	768
25– 29	906	902	622	794	316	620	862	908	326	712	332	646
30– 34	834	862	468	704	152	546	584	850	176	583	188	456
35– 39	790	842	116	620	30	416	456	820	78	478	108	252
40– 44	676	822	30	544	2	304	310	796	46	379	48	150
45– 49	484	806	14	448	2	224	236	750	24	302	16	78
50– 54	256	732	10	310	.....	112	136	682	14	224	4	26
55– 59	178	674	4	220	.....	32	56	634	6	175	2	8
60– 64	80	602	2	144	.....	8	12	550	2	72	.....	.....
65– 69	40	554	.....	78	.....	4	8	488	.....	36	.....	.....
70– 74	20	478	.....	24	.....	.....	4	360	.....	12	.....	.....
75– 79	12	400	.....	6	.....	.....	.....	304	.....	.....	.....	.....
80– 84	4	372	.....	.....	.....	.....	.....	260	.....	.....	.....	.....
85– 89	2	314	.....	.....	.....	.....	.....	224	.....	.....	.....	.....
90– 94	.....	286	.....	.....	.....	.....	.....	210	.....	.....	.....	.....
95– 99	.....	230	.....	.....	.....	.....	.....	188	.....	.....	.....	.....
100–104	.....	180	.....	.....	.....	.....	.....	170	.....	.....	.....	.....
105–109	.....	154	.....	.....	.....	.....	.....	142	.....	.....	.....	.....
110–114	.....	134	.....	.....	.....	.....	.....	96	.....	.....	.....	.....
115–119	.....	104	.....	.....	.....	.....	.....	82	.....	.....	.....	.....

TABLE 5—(*Continued*)

Temperature of development / Temperature of imaginal life	18° / 18°		18° / 25°		18° / 28°		28° / 18°		28° / 25°		28° / 28°	
Days	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
120–124	.....	78	.....	.....	.....	.....	.....	60	.....	.....	.....	.....
125–129	.....	68	.....	.....	.....	.....	.....	50	.....	.....	.....	.....
130–134	.....	52	.....	.....	.....	.....	.....	38	.....	.....	.....	.....
135–139	.....	30	.....	.....	.....	.....	.....	26	.....	.....	.....	.....
140–144	.....	18	.....	.....	.....	.....	.....	12	.....	.....	.....	.....
145–149	.....	8	.....	.....	.....	.....	.....	8	.....	.....	.....	.....
150–154	.....	6	.....	.....	.....	.....	.....	6	.....	.....	.....	.....
155–159	.....	2	.....	.....	.....	.....	.....	2	.....	.....	.....	.....
160–164	.....	2	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Mean	43.46 ±.40	70.61 ±.98	26.81 ±.24	40.96 ±.50	21.90 ±.21	30.67 ±.43	34.97 ±.37	65.25 ±.96	22.49 ±.27	35.75 ±.50	22.59 ±.27	28.52 ±.34
Standard deviation	13.08	32.53	8.129	16.57	6.909	14.03	12.33	31.809	8.981	16.34	8.862	11.12
C. of V.	30.10 ±.70	46.07 ±1.17	30.32 ±.70	40.46 ±1.00	31.54 ±.74	45.76 ±1.17	35.25 ±.84	48.75 ±1.27	39.93 ±.98	44.46 ±1.13	39.23 ±.97	38.98 ±.96
Absolute number of flies	493	499	501	497	497	493	496	496	499	492	491	493

* The figures in these columns give the number of survivors on the first day of the age interval indicated in the left-hand column of the table, headed "Days."

which is completely invisible in warm (28°) temperature females appears in a perfectly developed form only in flies reared at 18° C. Twenty-five degrees flies have only traces of black pigment in the seventh tergum.

IV

Table 5 presents the basic data of the experiments on duration of life. The numerators of the fractions heading the columns indicate the temperature during development, the denominators that during imaginal life. The number of survivors is calculated on the basis of 1,000 individuals at the moment of putting the flies in the one-ounce bottles.

The first point to which attention may be called is the difference between the sexes in mean duration of life. In all six series the females have a significantly longer duration of life than the males. The differences, with their probable errors, are shown in Table 6.

TABLE 6

DIFFERENCES BETWEEN THE SEXES IN MEAN DURATION OF LIFE

Series	Female mean minus male mean (days)	Ratio Diff./P.E. Diff.
18°/18°	+27.15 ± 1.06	25.6
18°/25°	+14.15 ± .55	25.7
18°/28°	+ 8.77 ± .48	18.3
28°/18°	+30.28 ± 1.08	29.4
28°/25°	+13.26 ± .57	23.3
28°/28°	+ 5.93 ± .43	13.8

From Table 6 it is obvious that all the sex differences are significant, by large margins. In the case where the two sexes are nearest together in mean duration of life (28°/28°) the difference between the two means is nearly 14 times its probable error. This result confirms what has been found in the earlier life table work on *Drosophila*. In the definitive life tables calculated from critically controlled *ad hoc* material for this form (Pearl

and Parker, 66), a sex difference in the same direction was observed in both wild type and vestigial flies, the difference being more marked in the case of the vestigials.

Regarding this matter Pearl (103) said: "It seems to be a rather general phenomenon, among groups of organisms in which there is sexual dimorphism, for males to be shorter-lived on the average than females, but statistically adequate quantitative information about duration of life is so meager that any such generalization would be premature at the present time. Such cases as those of *Dinophilus apatris* and various rotifers discussed by Korschelt, in which the male is dwarfed and obviously deficiently organized, and at the same time is short-lived, as compared with the female, are perhaps to be regarded as extreme illustrations of the dependence of duration of life upon bodily organization and pattern, a point which will be more fully discussed in later chapters. But these cases are not entirely probative evidence in support of a hypothesis that the organization or pattern differences implicate in normal sexual dimorphism of the kind and degree seen in man, for example, have generally as one of their normal expressions a shorter average duration of life in the male. Korschelt apparently inclines to the opinion that such a relationship is general, but makes the same point as is emphasized above, that the data available are insufficient to settle the question. It is of some interest to note that Blunck finds the average duration of life of female beetles (*Dytiscus*) greater than that of males, and also that Labitte's observations on the duration of life in beetles generally show the same thing."

The present experiments show that, in the case of wild type *Drosophila* the *sense* of this relationship between the sexes is not disturbed by the temperature at which they are reared or in which the imagoes live.

But from Table 6 it also plainly appears that the difference between the two sexes in mean duration of life

diminishes as the temperature during imaginal life *increases,* regardless of the temperature during development. This result is shown graphically in Fig. 5.

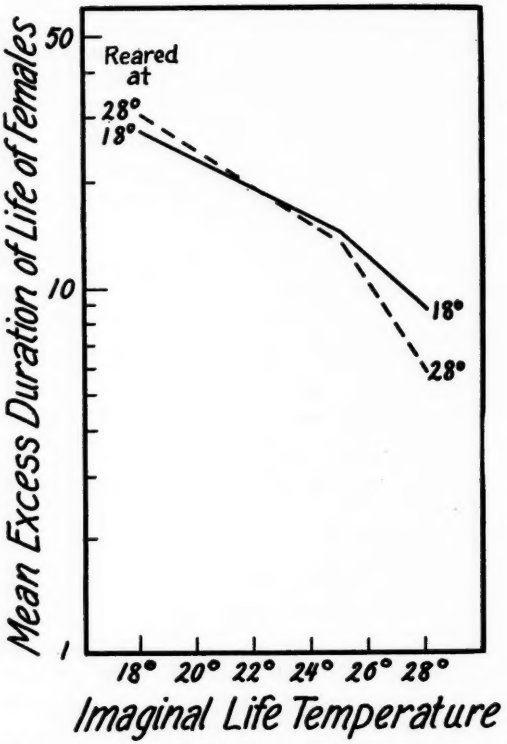

FIG. 5. Showing the decrease in the excess of female over male mean duration of life, with increasing temperature during imaginal life.

V

There are obviously two distinct questions which want discussion relative to the influence of temperature on duration of life. They may be formulated in the following manner:

1. What is the effect of the temperature during imaginal life upon the duration of life of *Drosophila?*

2. What is the effect of the temperature during development upon the subsequent duration of life as imago?

To answer the first question requires that the data be exhibited in such way as to show the change in mean duration of life associated with the same temperature during development but different temperatures during imaginal life. This is done in Figs. 6 and 7.

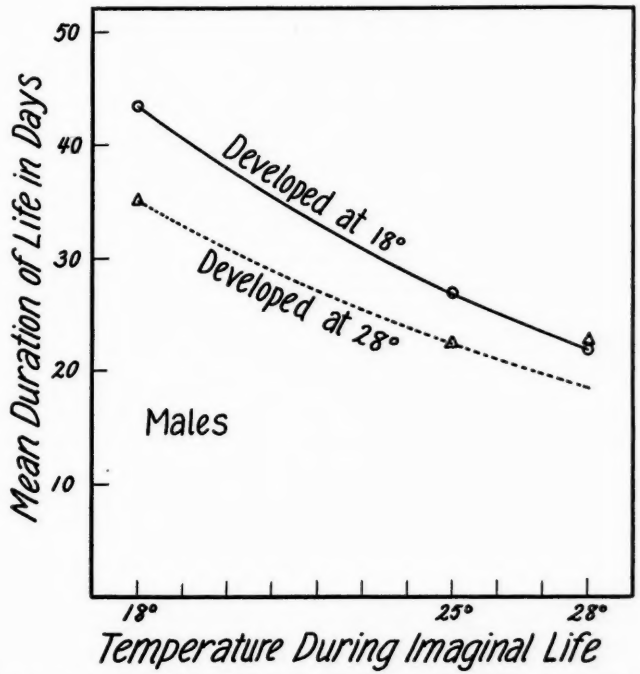

FIG. 6. Graph showing the average duration of life of males of *Drosophila* kept at different temperatures. The circles refer to flies developed at 18°, the triangles to those developed at 28°.

In Figs. 6 and 7 are shown the results of fitting to the observed mean durations of life given in Table 5 an exponential equation of the type

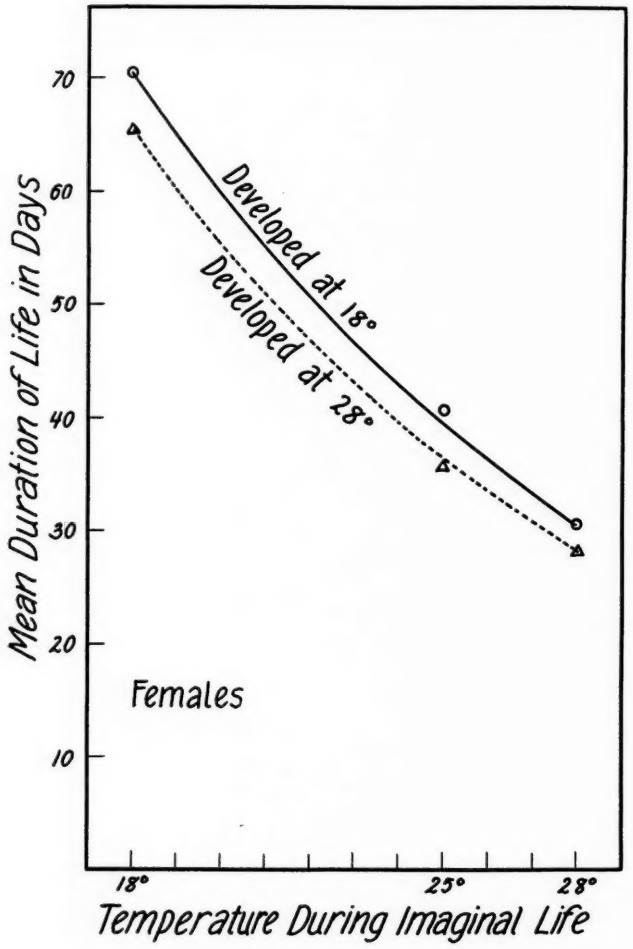

FIG. 7. The same as Fig. 6, but for females.

$$y = e^{a-bt°}$$

in which y denotes mean duration of life, and t temperature during imaginal life, in degrees Centigrade.

The equations for the males and females developed at 18° C., and that for females at 28° C., have been calcu-

lated on the basis of the observed durations of life at 18° and 28°. On account of the abnormally high duration of life of males in the series 28°/28° the equation for this series was computed from the observations at 18° and 25°.

From Figs. 6 and 7 it is apparent that the present experiments confirm the principal result of Loeb and Northrop's studies, namely that as the temperature during imaginal life is higher *Drosophila* lives, on the average, a shorter time. This is true of both sexes, and for both series of developmental temperatures.

The problem of a rational explanation of this temperature effect upon duration of life is an interesting one. The type of explanation which has been most in favor since Loeb discussed the matter may be characterized as basically a chemical one. Loeb and Northrop concluded that: "The observations on the temperature coefficient for the duration of life suggest that this duration is determined by the production of a substance leading to old age and natural death." The reasoning back of this conclusion is that temperature controls the rate of production of these hypothetical chemical substances.

An alternative explanation is biological in its essence, rather than chemical. It is a simple fact of observation that the total activity (movement, etc.) of *Drosophila* is greater in higher temperatures and less in lower temperatures. Now Pearl, in a considerable amount of recent work (*cf.* 103, and other publications there cited, and 104), and MacArthur and Baillie (101), have shown that total duration of life varies inversely as the rate of energy expenditure in living (growth, muscular movement, etc.). Wheeler (107) is of the opinion that in the inverse relation between rate of living and duration of life is probably to be found the physiological basis for the lengthening of the adult life in social insects. He points out that "all the subsocial and social insects live in small cavities of the soil or wood, in hives or, in the more exceptional cases of social wasps and certain trop-

ical ants, in the cavities of carton nests. The environment is, therefore, one which restricts or inhibits muscular movement and is dark, poor in oxygen, and of rather low and uniform temperature. All of these conditions would necessarily favor a lowered rate of metabolism and activity and an accumulation of fat in the insect body. The queens, or mothers of insect societies certainly impress one as having acquired their physiological and some of their morphological peculiarities as responses to just such an environment, for they are very sluggish and tend to lose the powers of flight (Meliponinae) or even the wings (ants and termites) and to acquire an accentuated anabolism as shown in the accumulation of fat and of yolk-laden eggs." Furthermore, Wheeler notes that: "Certainly the life-span of the three castes of ants and social bees would seem to be roughly proportional to their respective expenditures of energy."

It is in accord with the results of the body of carefully controlled experimental work cited in (103) and (104) to suppose that the reason why *Drosophila* has a shorter duration of imaginal life at higher temperatures is primarily simply because it is more active at those temperatures; or, in other words, has a higher "rate of living," and consequently a shorter absolute duration of life. To us such an explanation seems inherently more probable than the hypothetical chemical substances postulated by Loeb and Northrop, but we have no desire to press the point now, hoping with the passage of time to collect further experimental evidence which will make possible a critical quantitative discrimination between the two alternatives.

Figs. 6 and 7 show an interesting difference between the sexes in respect of the influence of temperature upon duration of life. The duration of life of the females is proportionately more shortened by high temperatures during imaginal life than is that of the males. Thus, taking flies developed at 18°, we see that whereas the

duration of life of the males living as imagoes at 28° is shortened below that of males living at 18° by 49.6 per cent. of the mean duration of life of the latter, the corresponding figure for the females is 56.6 per cent.

VI

Plotting the duration of life at different temperatures on arithlog paper we find that, with the exception of males in the 28°/28° series, the data give a straight linear distribution. We are therefore justified in concluding that within the temperature limits here used, the duration of life is an exponential function of the temperature. This result permits the calculation of the temperature coefficients commonly used in biochemical and biological literature, namely van't Hoff's Q_{10} and Arrhenius's μ. This was done (see Table 7) for our own material and for the material of Loeb and Northrop (see Table 8). Inspection of the values of Q and μ collected in Table 7 shows that there is a pronounced difference between the sexes in respect to these constants. The females have higher temperature increments than the males. Graphically it is seen in a sharper decline of the female curves of Fig. 7, as compared with the males shown in Fig. 6.

In general, the temperature coefficients obtained in this work are lower than those found by Loeb and Northrop for *Drosophila*. What the significance, if any, of this fact may be, we are not able to say. Our mean value for Q_{10} of 2.07 is at the classical spot for a purely chemical reaction in a homogeneous system, according to the *RGT*-rule as enunciated by van't Hoff. But it would be unwarranted, we think, to conclude from this fact either that duration of life is determined by a purely chemical reaction, or that *Drosophila* is a chemically homogeneous system. In fact we are tolerably certain that neither of these conclusions is, in fact, true, in spite of the value $Q_{10} = 2.07$. We are in agreement with the position taken by Höber (96) to the effect that great cau-

TABLE 7

TEMPERATURE COEFFICIENTS Q AND μ FOR THE DURATION OF LIFE OF *Drosophila melanogaster*

$$Q_{10} = \frac{K_{t_2}}{K_{t_1}} \quad \frac{10}{t_2 - t_1}$$

Sex	18°–28°	18°–25°	25°–28°
Flies developed at 18°			
♂	1.98	1.99	1.96
♀	2.30	2.18	2.62
Flies developed at 28°			
♂	1.77	1.88	.....
♀	2.29	2.36	2.12

$$\mu = \frac{\log K_{t_2} - \log K_{t_1} \cdot 4.605}{\frac{T_2 - T_1}{T_2 T_1}}$$

Flies developed at 18°			
♂	12005.7	11968.3	12096.1
♀	14607.7	13492.2	17299.8
Flies developed at 28°			
♂	7695.2	10936.5	.....
♀	14497.9	14907.5	13510.7

TABLE 8

TEMPERATURE COEFFICIENTS Q AND μ FOR THE DURATION OF LIFE OF *Drosophila melanogaster*

Series		Q	μ
18°–28° (our material summed up)		2.07	12717.3
Loeb's data	10°–20°	2.99	17894.1
	15°–25°	3.24	20866.4
	20°–30°	2.96	20556.5

tion must be used in drawing biological conclusions from the numerical values of temperature coefficients.

VII

We may turn now to the second problem stated above, namely the effect of the temperature during embryonic, larval and pupal development upon the duration of imaginal life. The data for mean duration of life from Table 5 *supra* are so arranged in Table 9 as to give the answer. The results are shown graphically in Figs. 8 to 13.

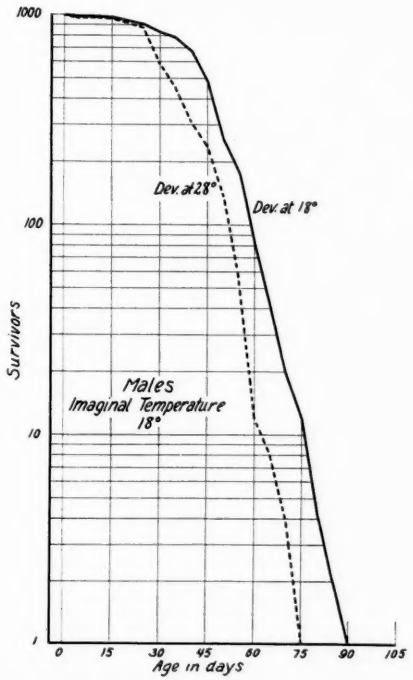

FIG. 8. Survivorship lines of males kept during imaginal life at 18° C. In this figure, as well as in Figs. 9, 10, 11, 12 and 13, the continuous line represents flies developed at 18° C., the broken line those developed at 28° C.

From Table 9 and the diagrams it is, in the first place, apparent that the temperature at which development (embryonic, larval and pupal) takes place affects the

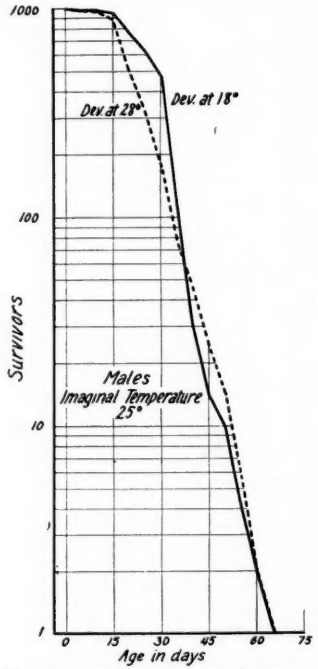

FIG. 9. Survivorship lines of males kept during imaginal life at 25° C.

TABLE 9

THE EFFECT OF TEMPERATURE DURING DEVELOPMENT UPON THE DURATION OF IMAGINAL LIFE AT THREE CONSTANT TEMPERATURES

Temperature during imaginal life	Mean duration of life of flies reared at 18°	Mean duration of life of flies reared at 28°	Difference	Diff. / P.E. Diff.
		Males		
18°	43.46 ± .40	34.97 ± .37	+8.49 ± .54	15.7
25°	26.81 ± .24	22.49 ± .27	+4.32 ± .36	12.0
28°	21.90 ± .21	22.59 ± .27	− .69 ± .34	2.02
		Females		
18°	70.61 ± .98	65.25 ± .96	+5.36 ± 1.37	3.91
25°	40.96 ± .50	35.75 ± .50	+5.21 ± .71	7.3
28°	30.67 ± .43	28.52 ± .34	+2.15 ± .55	3.9

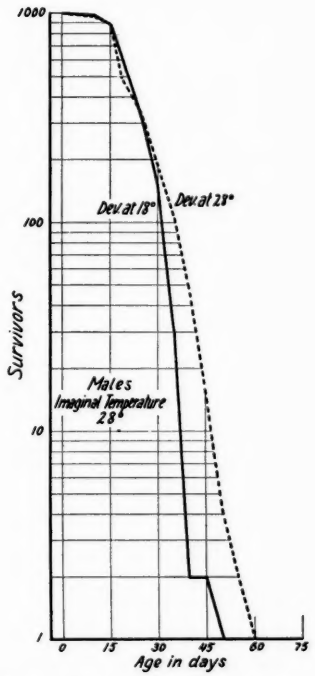

FIG. 10. Survivorship lines of males kept during imaginal life at 28° C.

duration of subsequent imaginal life, regardless of the temperature at which the latter lived. A high temperature (28°) during development shortens the duration of the subsequent imaginal life at all temperatures and in both sexes. The only exception to this statement in the whole experience is the male 28° imaginal series. The males in the 28°/28° series gave an abnormally high mean duration of life.

It is of some interest to compare the relative changes in body size and in duration of life produced by different temperatures during development. From Tables 3 and 4, taking the four most reliable measures of body size, femur and tibia lengths and wing length and breadth,

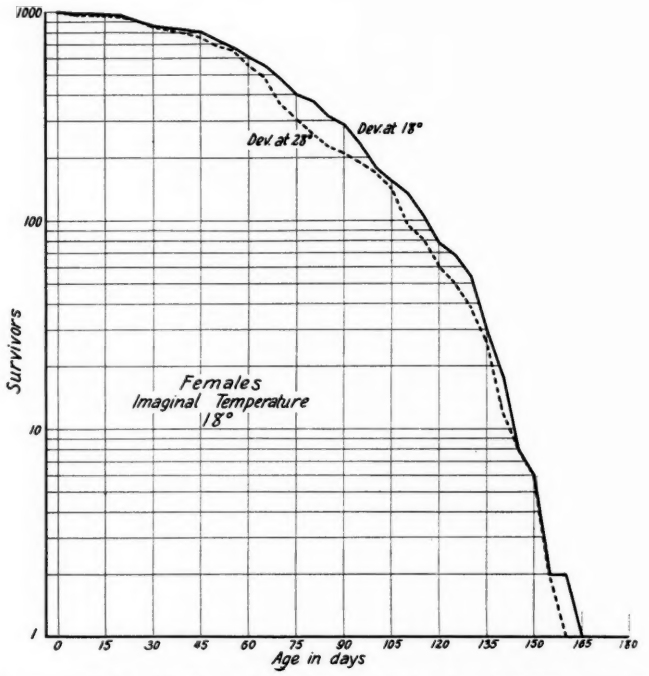

FIG. 11. Survivorship lines of females kept during imaginal life at 18° C.

we derive the following percentages regarding reduction in *body size.*

Character	Percentage which difference between 18° and 28° series is of 18° flies	
	Males	Females
4 (femur length)	7.0	5.7
5 (tibia length)	6.9	6.0
8 (wing length)	13.4	10.0
9 (wing breadth)	13.1	9.4
Mean of four characters	10.1	7.8

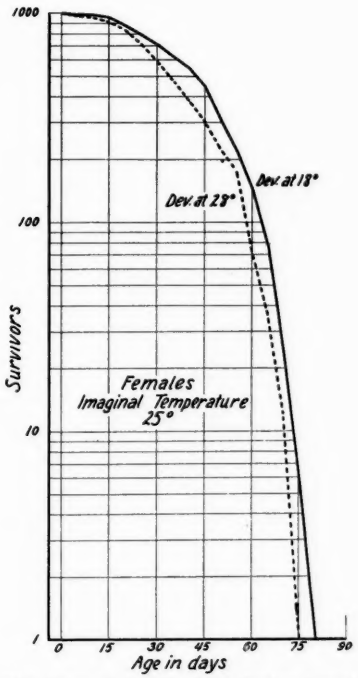

FIG. 12. Survivorship lines of females kept during imaginal life at 25° C.

It is evident that wings are proportionately more reduced in size than are the legs in high temperature as compared with low temperature flies. Furthermore, the reduction in size as a whole (average of four measurements), as well as in each separate character of the four, between 18° and 28° flies, is greater in males than in females.

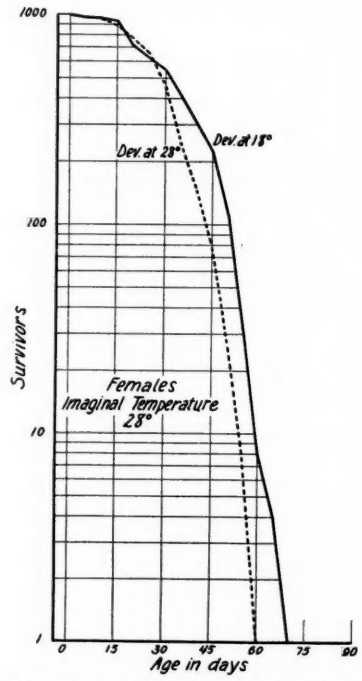

FIG. 13. Survivorship lines of females kept during imaginal life at 28° C.

Corresponding percentages for *duration of life* of flies reared at 18° and 28°, respectively, are as follows:

Temperature during imaginal life	Percentage which difference between flies reared at 18° and 28° is of 18° flies	
	Males	Females
18°	19.5	7.6
25°	16.1	12.7
28°	.....	7.0
Mean of all series	17.8	9.1

From these figures it is seen, first, that the reduction in mean duration of life resulting from rearing the eggs, larvae and pupae at 28° as compared with 18°, is some-

what greater proportionally than the corresponding reduction in body size, but still the two sets of figures are of the same general order of magnitude. Furthermore, it is apparent that proportionate reduction in duration of life as a result of high temperature is greater in males than in females, just as it is in body size.

These results indicate with a considerable degree of probability that in these experiments the quantitative effects of temperature differences upon the biological processes concerned in growth are of approximately the same order of magnitude as the quantitative effects of temperature differences upon the biological processes concerned in the determination of duration of imaginal life. This is the kind of numerical result which would be expected on the rate of living theory of life duration, because in both cases the effect of increased temperature is to speed up the *rate* of the biological processes involved. In the 18° flies we have a *slow rate* of energy expenditure in growth and during imaginal life (flies very inactive), and we should therefore expect on the theory the lengthened duration of imaginal life which we observe. In the 28° flies there is a short developmental period and a consequent *rapid rate* of energy expenditure during growth, and during imaginal life (flies very active). This leads to the expectation of a short duration of imaginal life, which is in fact observed.

VIII

Summarizing, it has been shown in this paper that:

1. *Drosophila* reared at 18° C. is distinctly larger in a series of bodily dimensions than when reared at 28° C. Furthermore, the pigmentation is different in the two cases, notably with reference to the pigmentation of the seventh tergum.

2. Females were longer lived than males in all series of these experiments. The difference in duration of life between the two sexes diminishes as the temperature

during imaginal life increases, in these experiments, regardless of the temperature during development.

3. As the temperature during imaginal life increases the duration of life decreases. The relationship between duration of life and temperature is exponential.

4. For the temperature range 18° to 28° used in these experiments the temperature coefficient for duration of life had, as average figures, the following values:

$$Q_{10} = 2.07$$
$$\mu = 12717.3$$

5. The relative or proportional influence of temperature upon body size and upon duration of life was, in these experiments, of the same order of magnitude. This fact furnishes confirmatory evidence to the theory that an important factor in determining the duration of life is the rate of energy expenditure during life.

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THE MECHANISM OF CILIARY MOVEMENT[1]

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SINCE their discovery in 1834, ciliated cells have been shown to occur in nearly every group of the animal kingdom. Fine hyaline processes project from the surface of the cells and vibrate in such a way as to exert a resultant pressure on the surrounding medium, the pressure being maintained, of course, in a definite direction. The vibratile structures are seldom more than one five hundredth inch long and their diameter is usually less than one ten thousandth of an inch; in many cases the observed dimensions are much less, for cilia may be one two thousandth of an inch in length and have a cross-section of not more than one one hundred thousandth of an inch. When observed under a microscope, most cilia appear to be moving very rapidly, although in fact the velocity of their movement never exceeds thirty feet per hour (or one mile per week!). An example may perhaps be useful; if a cilium is thirty microns in length and if its tip pass through an arc of 180° twenty-four times per second, then the distance travelled by the tip in one second is $\pi.30 \times 24$ microns per second, which is approximately twenty-seven feet per hour.[2] The apparent high velocities observed under the microscope are partly due to the fact that the microscope magnifies linear dimensions but does not affect the dimension of time; in addition to this is the inability of the eye to focus clearly a series of events which occur within the short space of time occupied by one ciliary beat.

Inasmuch as a cilium represents a very thin thread moving in a viscous medium at low speed, it constitutes

[1] A lecture delivered at the Marine Biological Laboratory, Woods Hole, August 11, 1928.

[2] See Bidder, G. P. (1923) *Quart. Jour. Micro. Sci.* 67, 293.

a hydrodynamical system which has yet to be investigated as thoroughly as is desired. As is so frequently the case, the biologist is dealing with structures whose small dimensions involve controlling factors which are not the same as those applicable to larger objects. Stokes' law, which defines the resistance encountered by a small spherical body moving through water at a low speed, differs materially from the laws which define the resistance encountered by a large fish or by a torpedo moving at high speed. Until certain hydrodynamical problems have been submitted to purely physical analysis a true understanding of ciliary movement will elude us, and biologists will be forced to do what they can with the limited data available. If discretion were the better part of valor I would state the biological facts and leave you to digest them if so inclined. I propose, however, to present the facts against a theoretical or speculative background, largely in the hope that some part of the picture may induce a physicist to cooperate in what is to me a fascinating subject.

When subjected to the usual methods of fixation and staining all ciliated cells exhibit much the same appearance. The vibratile elements appear as a series of filrils bearing, as a rule, very little resemblance to the living organ: at the base of the cilium or flagellum there is always a granule or series of granules possessing a high affinity for basic stains. From these basal granules there may or may not project a series of intracellular filrils, which personally I have never seen in the living cell. Many attempts have been made to base a conception of the ciliary mechanism on the morphology of permanent preparations; none of them have stood the test of physiological analysis.

Starting from first principles we may regard all vibratile organs of locomotion as propellers setting in motion a current of water in a definite direction. There are two main types—the paddle and the screw, both of which occur in nature. Cilia may be regarded as paddles,

whereas one type of flagellum is essentially a screw. In order that an object may be propelled by means of a paddle it is essential that the thrust imparted to the water during the effective stroke should be greater than that during the recovery or reverse stroke. A paddle or a cilium whose forward motion is precisely the same as the backward stroke can not act as a propeller, although it may set up an oscillating disturbance in the water. An examination of certain cilia which are known to act as efficient propellers is possible if the frequency and velocity of their beat be reduced by experimental means. By exposing the frontal cilia on the gills of *Mytilus* to sea water containing a limited excess of CO_2 the speed of beat rapidly falls and the details of the movement can be studied. During the effective stroke the cilium is seen moving forward as a rigid rod with its full surface exposed to the water; during the recovery stroke, however, the cilium moves back in a flexed condition whereby considerably less surface is exposed to the resistance of the water. The recovery stroke is thus effected by the transmission of a bend which starts at the base of the cilium and travels to its tip. Moving cilia of this type have been shown to occur in many groups of animals and may perhaps be the most common type of vibratile propeller. The simplest mechanical model of such movement is provided by a strip of curved steel wire fixed at one end. If the wire is deformed by means of a stop, which not only bends the steel but also travels from one end to the other, the form of the wire conforms to that of a cilium during the recovery stroke. On releasing the wire from the stop, the former flies forward along a path corresponding to the effective beat. Like all mechanical models of biological structure this conception of a cilium must be used with caution, since the speed of the effective beat is, unlike that of the recoil of a bent wire, a variable quantity. The model serves to illustrate, however, that a cilium can act as a propeller if a "bending" wave passes along its length from base to tip, and that kinetic energy

is liberated during the recoil. That a cilium stores potential energy when it is bent is clearly seen when a stationary cilium is mechanically deformed by needle. On removing the needle, the cilium rapidly regains its normal form, showing clearly that it is composed of elastic material.

In the case of a typical cilium, the whole filament at the end of the recovery stroke is subjected to a more or less uniform bending force, and the axis of bending coincides with or is in the same plane as the long axis of the cilium. In a typical flagellum there are two essential differences. Firstly, the propagated bending waves are of short wave length—so that more than one wave is present at any instant. Secondly, the axis of bending is often inclined to the long axis of the flagellum. The propulsive power of the flagellum was attributed by Bütschli to the forward component of the force which acts on the water at right angles to the lateral oscillations of each element of the flagellum. Such a conception appears to ignore the fact that if the wave is symmetrical about the axis of locomotion these components must be equal and opposite to each other. In order to effect propulsion it is essential that the bending waves should travel along the flagellum and should always travel in the same direction. The propelling force is equal to that which would be exerted by the projection of a series of permanent "humps" or waves, equal in size, form, velocity and frequency to the moving waves which are actually observed. In all cases the organism moves in the opposite direction to that followed by the waves—so that if the waves travel clockwise round a line coincident with a prolongation of the longitudinal axis of the organisms, the latter itself rotates in an anti-clockwise direction as well as moving forward through the water.

Van Trigt observed that as the activity of the flagellated cells of *Spongilla* declines, so the waves passing along and around the flagellum alter in form. Fast movement with strong propulsive power is characterized

by waves of short wave length and low amplitude; as movement becomes less active, so the wave length and amplitude increase. This is precisely what one would expect to observe if the waves represent regions of the flagellum which are storing and releasing potential energy in a manner comparable to the bending and releasing of an elastic filament. If a straight strip of steel wire is subjected to uniform bending force (f) along its longitudinal axis the strip will bend into the arc of a circle whose radius (R) is equal to EK/f where E is Young's modulus and K is the moment of inertia about the neutral axis of the wire. If, however, the same bending force is applied about an axis which is inclined at an angle (ϕ) to the longitudinal axis of the wire, the latter will bend into a regular helix whose generating cylinder will be equal to $2R$ and whose pitch is $2\pi R \tan (90° - \phi)$. In terms of flagellar movement these two expressions represent the amplitude and wave length of the flagellar waves. Hence the amplitude of the waves is $\frac{E.K.}{f}$ and their wave length is $\frac{2\pi E.K. \tan (90° - \phi).}{f}$ In other words, as the bending force becomes less intense, so the amplitude and wave length of the disturbances passing along the flagellum will increase. In this way we come to regard both ciliary and flagellar propulsion as the result of propagating bending forces along the length of the vibratile structures.

At this point we may inquire whether the mechanical energy stored in the cilium or the flagellum arises as such in the body of the cell or whether it is generated in the filament itself. If a cilium or flagellum is detached from the cell distally to the basal granule, it is usual to find that all movement instantly ceases; from this one might imagine that the mechanical power of the cilium originates as such in the cell and is associated with the basal granule. Such a view, however, meets with strong theoretical objections and is incompatible with certain types

of movement which actually occur. If a bending wave by traveling along an inert flagellum propels an animal against the viscous resistance of water, the wave must lose energy as it travels, and must therefore alter in form as it progresses. If the figures given by Riechert for the flagellum of *Spirillum* are correct, no such changes in form occur, and we must therefore assume that the wave is gathering energy as fast as it is expending it. Exact analysis of the form of the waves is now being attempted by cinematographic methods and it is hoped that definite information may soon be available. In the meantime there are some cases which show fairly clearly that the mechanical energy stored by a moving flagellum arises as such in the flagellum itself. In certain protozoa the distal end of a flagellum may exhibit active movements, whereas the proximal regions remain at rest. Also in some cases the waves arise at the distal end of the flagellum and pass down towards the cell. In such cases the flagellum must be looked upon as an active unit capable of generating tension energy from chemical energy at all points along its length. Presumably a bending wave passes along a cilium or a flagellum much as a contraction wave passes along a muscle fibre. From a mechanical point of view we may look upon ciliary movement as essentially the same as muscular movement, except that in the latter case the disturbances in form are set up parallel to the long axis of the fibre, whereas in the former the disturbances are developed along the transverse axis. An analogy is provided by a stout length of rubber: it can store energy by being stretched or store energy by being bent; in both cases the essential nature of the process is the same.

How can a cilium generate along its longitudinal axis a bending force such as has now been described? Schäfer suggested that a cilium is a hollow tube with one side more extensible than the other, and by means of a "protoplasmic pump" water is driven into the tube, thereby causing the more extensible side to become convex; on

pumping water out of the cilium the latter straightens once more. Against this conception there are many objections, but it includes two valuable features: (1) the suggestion that the bending of a cilium is due to a redistribution of water within the active cilium and (2) that both effective and recovery strokes are active processes —since the speed of the effective beat depends on the rate of removal of water and not solely on the mechanical properties of the cilium as a whole.

Mechanical models of physiological processes are dangerous inventions, but they may have their uses. If one side of a straight cilium becomes capable of absorbing more water than the other, then the cilium will bend into the arc of a circle, the hydrated side being convex. If the water then redistributes itself equally between the two sides, the cilium will straighten out. In bending, the elastic cilium will store potential energy and will release this as kinetic energy when it straightens. A simple model can be made from a strip of paper cut from the page of *The Collecting Net* or most types of note paper. If a strip about one fourth inch wide be cut from the top of a page and moistened on one side, the strip bends about its longitudinal axis. It straightens again as the paper dries or the water diffuses equally across the thickness of the paper; if, however, a strip is cut along the diagonal of the page, then, on moistening the one side, the strip curves into the form of a helix because the fibres of paper which absorb the moisture are no longer orientated at right angles to the longitudinal axis of the strip but are inclined at an angle to it. How can such a change in the distribution of water be brought about in the living cilium?

Such a reorientation of water would occur if, along one side of the cilium, there existed an ionized colloidal system whose affinity for water depended on its degree of ionization. If, for example, there is a series of protein molecules all on the alkaline side of their isoelectric point and there is generated at their surface a number

of hydrogen ions, then the affinity of the protein for water will fall and the system will contract. If the hydrogen ions are now removed, water will return to the protein and the cilium will straighten out. The forces involved by such changes are very great, and we may perhaps accept the model as a working hypothesis of ciliary movement.

Unfortunately, it has so far proved impossible to put this theory to experimental test, but we can get some indication of its validity by indirect methods. If ciliary movement involves a rhythmical change in the ionization of some intraciliary surface it ought to be possible to alter the speed and nature of the beat by reagents which are known to alter the ionization of such systems. Before doing so, however, it is necessary to look upon the whole problem from a wider point of view.

The final result of ciliary movement involves an expenditure of energy by the cell, and in the long run this energy must come from the chemical energy stored in the cell itself. What, then, are the intermediate steps? The whole system, like most biological phenomena, has proved to be very complex, but so far it seems possible to divide the energy cycle of ciliary movement into five separate phases. Firstly, the cells must contain a supply of material capable of maintaining ciliary activity for a prolonged time—since under suitable conditions an excised fragment of ciliated epithelium will remain active for many days. Attempts to isolate the material which is supplying the energy for movement have not been very successful. Quite recently Mr. Boyland kindly analyzed the gills of *Pecten* and found small traces of glycogen which disappeared from excised fragments during a period of ciliary activity: it seems doubtful, however, whether the small traces of carbohydrate present in the cells can constitute the sole source of ciliary energy; but whatever be its nature we may safely assume that some such "ultimate reserve" of ciliary energy is actually present. If we watch a fragment of excised epithelium,

sooner or later the speed of the cilia begins to fall and finally movement ceases; nevertheless, the ultimate reserve is not exhausted because by mechanical stimulation a new outburst of movement occurs. Certain reagents have been found to be peculiarly effective in calling forth such new and prolonged outbursts of movement. Thus with the lateral cilia on the gills of *Mytilus*—movement usually ceases after one to two hours in normal sea water. If, however, we rob the sea water of some of its magnesium, or if we increase the concentration of potassium or, best of all, add a trace of the drug veratrin, the lateral cilia quickly begin to beat, and on transference to running sea water will maintain their activity undiminished for as much as twenty-four hours. These reagents therefore seem to "recharge" the cell with a source of energy which is absolutely essential for movement, and which is derived from the ultimate reserve. This second compound we may call the Immediate Reserve of energy or the fuel which is used by the ciliary machine. It may be worth noting that those reagents which "recharge" exhausted cells also accelerate the formation of lacticogen from glycogen in a muscle cell. The third step in the ciliary cycle can be detected by exposing cells to $H^{\cdot}$ or to a reduced concentration of $Mg^{\cdot\cdot}$; under either of these conditions an instantaneous change occurs in the rapidity of the ciliary beat; by adding $H^{\cdot}$ the beat slows down and stops; by reducing $Mg^{\cdot\cdot}$ the beat quickens up. Each change is reflected in the O_2 consumption of the cells and it looks as though we were operating on the throttle of the ciliary machine, determining how much active substance is getting to the sensitive sites per unit time. I am inclined to think that it is this reaction which controls the rate at which an acid is liberated at a protein or other colloidal surface, for it is certainly the reaction which immediately precedes the bending of the cilium. The fourth step involves the actual mechanism of bending: if we imagine the bending as due to the liberation of $H^{\cdot}$ at an active surface, then the recoil occurs because

the $H^{\cdot}$ is removed, possibly by neutralization by the surrounding matrix. Bending only occurs if there is a supply of energy and if calcium is present. In the absence of calcium the cell continues to dissipate energy and consumes its full quota of oxygen, but no movement occurs; further, there must be a minimum quantity of water in the cell. This indicates that the active surfaces which cause the cilium to bend contain calcium and are possibly a basic calcium salt of a protein, which, in the presence of acid, loses its affinity for water and so contracts. In the absence of $Ca^{\cdot\cdot}$ the redistribution of water can not occur, and the throttle being open the engine runs without doing any useful work. Finally, if the cell is deprived of oxygen, ciliary movement will continue for about forty-five minutes at room temperatures, after which movement ceases, to be resumed again after a definite period if oxygen is again available. Ciliary motion thus appears to be an anaerobic process, but oxygen is required for the removal of the products of activity.

In many respects the whole cycle is similar to that of a muscle fibre, and we may continue to look upon the two types of contractile processes as having the same fundamental nature.

So far we have dealt solely with the individual cilium, but a cursory examination of most ciliated epithelia reveals the fact that each cilium is not beating independently of its neighbors. Any particular cilium is slightly in advance of that behind it and slightly behind the one in front, and this gives the well-known effect of the metachronal wave. The mechanical effect is, of course, to give a steady flow of water or a steady rate of progression; if all the cilia beat in unison, movement would be discontinuous. The nature and properties of the metachronal wave are, however, extremely obscure, and so far no real analysis is available. Grave and Schmidt have, it is true, described a series of intercellular filrils which they regard as a coordinating mechanism for the latero-frontal cilia of *Mytilus,* but there is some doubt

I think about the validity of their conclusions. Bhatia failed to confirm their observations. Here again the problem can be approached by experimental methods. Firstly, it can be shown that flagellated cells can synchronize their movements without any organic connection being established. This occurs in spermatozoa or in *Spirochaeta balbianii* when the anterior ends of individual cells come into contact with each other. In the case of epithelia, however, the cells are not synchronized but are obviously controlled by a timing mechanism which may be located in the ciliated cells or in some other layer of the tissue. In looking for the seat of this timing mechanism it is useful to bear in mind certain facts. First, every isolated ciliated cell is normally in active motion; secondly, the most frequent type of extraneous control to which cilia are subjected is of an inhibitory nature. This is particularly well seen in the velar cilia of the Molluscan veliger larva. These cilia exhibit alternating periods of activity and rest. Dr. Carter has shown that if the velar nerve be cut or if the larva be exposed to anesthetics of sufficient concentration to inhibit the nervous impulses, the velar cilia continue to beat without intermission. Similar inhibitions are known in the case of *Ctenophore* cilia. As far as is known there are no cilia which are only active when stimulated—such cases only occur in the case of moribund tissues, with the possible exception of the cilia on the tips of the snail *Physa*. How far the power of responding to the inhibitory stimuli plays a part in determining the metachronal rhythm is unknown. The rhythm itself is one of the most interesting phenomena associated with ciliary activity. It may move in the same direction as the effective beat, it may move against the effective beat or it may move at right angles to it, but for any given tissue the direction is always the same.

We may now perhaps consider the place of ciliated cells in the general economy of nature and try to get some picture of ciliated life. Consider, for example, any

small ciliated organism moving through water. Practically the whole of its energy is used in overcoming the viscous resistance of the medium. Its motive power in terms of muscular units is low and its maximum velocity is also low, but if the animal is quite small, then it can move at a reasonably rapid speed in terms of its own size. Thus a spermatozoon is moving about twice as fast as a fish if we reckon velocities in terms of the organism's own length, but in absolute units the fish is moving ten thousand times as fast. Slow speed has its compensations, for thereby the kinetic energy of the moving organism is small. In other words, a ciliated organism starting from rest with its full ciliary power at work very rapidly attains its full maximum speed, whereas a larger organism of higher specific gravity takes some little time to reach its maximum speed. Similarly, if the kinetic energy during motion is low, then as soon as the organism shuts off its ciliary power the animal comes almost to a dead stop—the distance traveled being given by the equation:

$$\frac{\text{Velocity} \times \text{Mass}}{\text{Resisting Force due to Viscosity}}$$

For a typical ciliated organism, the animal comes to rest within one twentieth of its own length. There is no need for any braking mechanism. Further, if its specific gravity is low, a ciliated organism can travel with equal facility in any direction. For moving heavy bodies or for moving bodies at a really high speed cilia are useless, for they do not develop sufficient horsepower.

There are, however, two functions for which cilia are peculiarly useful. Firstly, they can drive a film of water over a living surface without involving any change in the form of the surface. Thus the bronchial and nasal passages of man are continuously cleansed by the action of their ciliated epithelia. The mucus which collects at the back of the throat during catarrh is accumulated

there by the bronchial cilia, just as the food of *Mytilus* is collected at the mouth by the branchial epithelia.

The second function peculiar to cilia is the maintenance of a liquid current through narrow tubes at low pressures. As long as the tube is narrow, the cilia are efficient—as the tube widens, the cilia are unable to exert sufficient action on the inner layers of fluid and circulation is only possible at the higher pressures induced by muscular action. No ciliated current has a pressure of more than 4 mm of water—which illustrates how ineffective would be the effort of cilia to maintain the blood circulation of a large vertebrate animal. Yet in the world of invertebrate animals, where velocity of movement is low and where the habits of life are quiet, cilia play a most important and sometimes spectacular rôle. As Dr. Bidder has remarked, "by the waving of hairs one one-thousandth inch in thickness at a mean speed of seven feet per hour a single specimen of the sponge *Leucandra* passes through its body a ton of water in six weeks."

In conclusion I would like to refer for a few moments to the phenomena of ciliary reversal in the *Metazoa*. Until fairly recently a reversal of the ciliary stroke was believed to be a fairly common phenomenon. Since, however, no propulsion can occur if the form of the effective and recovery strokes are the same, it follows that ciliary reversal must involve a reorganization of the ciliary machinery. One by one the examples of ciliary reversal have been shown to be the result of antagonistic currents set up by different tracks of cilia, the intensity of each track being controlled by muscular movements which interfere with its effectiveness. Under one set of conditions a particular track will be enclosed within temporary walls of tissue which are erected by local muscular contraction, thereby removing the cilia from the sphere of action. Under other conditions this track will be exposed and the other covered in. Almost the only remaining example of ciliary reversal remaining in the

Metazoa is the result of the beautiful observations of Dr. Parker on the oral disc of *Metridium,* where the current is reversed when the disc is exposed to food material. To any one interested in the mechanism of the cilium itself this case is of extreme interest, and knowing Dr. Elmhirst, of the Marine Station at Millport, to be interested in the feeding mechanism of anenomes I asked him to describe this process in an English species of *Actinoloba.* He at once presented me with a reprint from which I quoted in a book as follows: "Longitudinal groves run down the gullet, and when food is being swallowed the inflow is along the groves; conversely a ciliary outflow runs up the ridges." It therefore seemed to me to be just possible that this is what might occur at Woods Hole. On my arrival here I found Dr. Parker with my book in one hand and a *Metridium* in the other, and one of the most pleasant experiences I have had in Woods Hole has been the convincing demonstration by Dr. Parker that a true reversal of the same ciliary current does actually occur. How the cilia do it we do not know; unfortunately they are very small; one is loath to think of a double ciliary machine only one half of which is in operation at once and perhaps it is just possible to imagine that the reversal is due to a change in the "tone" of the cilia. For such a suggestion there is some slight experimental evidence.

THE MYSTERY OF *ALABAMA ARGILLACEA*

GEORGE N. WOLCOTT

ESTACION EXPERIMENTAL AGRICOLA, LIMA, PERU

BEFORE the advent of the boll weevil in the cotton fields of the south, the most destructive of pests attacking this crop was the cotton leaf caterpillar, *Alabama argillacea* Hübner. Although its relative importance is not now so great, it still continues to strip cotton plants of their leaves, leaving only their bare stalks and nearly matured bolls undevoured in its sweep across the cotton belt. We now know that this pest is relatively easy to control, and in fact that its apparent destructiveness may at times in reality be beneficial in stimulating the early maturing of the bolls and in rendering picking easier because the leaves have been removed. But in the early days of economic entomology, this pest well deserved the attention given to it, and the first extended account of these investigations is rarely equaled, even at the present time. Historically, *Alabama* is interesting because in poisoning its caterpillars paris green was first extensively used as an insecticide (following its use against the Colorado potato beetle), and indeed until recently continued its popularity as a means of chemical control.

Despite the careful and extensive initial investigations on this insect, much yet remains to be learned concerning it. Indeed, only gradually after this first extensive report did the data begin to accumulate out of which we piece together what we think we know regarding its habits. After a succession of waves of destructive caterpillars has swept across the fields during the summer months, nothing more is to be seen of the pest in the cotton fields. But early in the autumn, fruit growers in northern Ohio complain of grapes and other ripe fruits being injured by sleek grayish-brown moths, which rasp

their skins with their barbed proboscis, and eagerly suck up the exuding fruit juice after their flight of hundreds of miles from the south. These moths are the adults of the cotton caterpillars, and a few days or weeks later, swarms of them are reported about electric lights in the most northern cities of the United States, and even in Canada. They are hundreds of miles from anything on which their larvae will feed, and represent as futile and self-destructive a gesture as the Children's Crusade.

The earliest investigators fruitlessly searched in all possible locations, especially in the south, for the place in which the moths spent the winter, and only gradually did it appear probable that *Alabama* never hibernates in the United States, and possibly never hibernates at all, but that the first outbreak of the caterpillars each summer in the most southerly of cotton fields represents a new, fresh and entirely separate invasion of the moths from the tropics. This naturally raises the question of where in the tropics does *Alabama* occur the year round —or whether there is any place in the tropics where it does occur at all months of the year, for if there is such a place, why then does it annually invade the cotton fields of the United States? The answers to none of these questions is known, and the present note is merely to raise the questions and to present some observations that may aid in their solution.

Cotton production in tropical America is by no means extensive. The cotton grown in Mexico is mostly on the high central plateau where the winters are as cold as in the southern United States, and quite as inimical to *Alabama*. Cuba is so exclusively interested in sugar-cane and tobacco that only the most diligent search discloses a few fields of commercial size. Jamaica has a more diversified agriculture, but cotton occupies a scarcely more prominent place. In southern and central Haiti, however, cotton is of very decided importance, and ranks second in value of the exports of the republic. Yet Haiti is not the winter home of *Alabama*. Although

cotton plants grow there during the entire year, the commercial crop matures in the months of December to February, but even before the first bolls begin to open, *Alabama* caterpillars have disappeared, not to reappear until June. In northern Haiti, where winter rains delay the production of bolls and cause fresh leaves to appear, the caterpillars nevertheless disappear early in the winter, and none are to be found for the next six months. In Santo Domingo, cotton is little grown, and in the scattered cotton fields in limited areas in Porto Rico, *Alabama* sometimes appears in destructive abundance, but in other years is not to be seen. *Alabama* has not a modest and retiring disposition, and if it occurs at all is present in such abundance that there is no doubt about it. Individual and scattered volunteer plants are rarely attacked, and it would appear that the moths recognize nothing smaller than an entire field of cotton as a place for laying their eggs. Thus one need not consider the possibility of a few scattered individuals escaping observation, for the nature of *Alabama* is to exist only in crowds, and if one finds a few caterpillars on scattered cotton plants, they invariably prove to be another species of cotton caterpillar: *Anomis doctorium* Dyar. Cotton is rather extensively grown in several of the Lesser Antilles, but, if one analyzes the records, he will find no indication of its presence throughout the year, or even of outbreaks every year. Indeed, all available evidence indicates that, so far as Mexico and the West Indies are concerned, each appearance of *Alabama* in their cotton fields is just as truly a new and separate invasion from without their territorial limits as is its appearance in the temperate zone cotton belt of the United States.

Few observations have been made on cotton in Central America and in the northern republics of South America, and practically nothing is specifically known of *Alabama* in this region, but this is largely due to the fact that there is little to record. Cotton is grown in Venezuela at Valencia, according to Mr. Luis Montero Bernales, and

what is presumably *Alabama* does occur there in typical infestations, but as the cotton depends entirely on seasonal rainfall for its growth and can be grown at only one time of year, this surely is not the place to look for a year-round occurrence of its plague. Cotton is extensively grown in Brazil, and presumably under such a wide variety of conditions and over such an extended territory that it is possible that *Alabama* may here find conditions suitable for existence during every month of the year. Dr. C. H. T. Townsend assures me that such is the case, yet unless specific observations are made on this particular point, it is quite possible that the intensity of its outbreaks at some seasons of the year may obscure the fact that it is not present at other times.

In the absence of such observations, it seems worth while to record certain notes on the occurrence of *Alabama* in Peru which indicate a most striking, but reversed similarity to its habits north of the equator. In all that will be said regarding the distribution of *Alabama* in Peru, only the coast has been considered, for away from the coast and the chilling influence of the Humbolt current, on the other side of the Andes, normal tropical weather conditions exist, and wherever cotton is grown, *Alabama* is recognized as the invariable accompaniment of its cultivation on a large scale. Although politically part of Peru, in other respects this region is comparable to the cotton-growing districts of Brazil.

A cotton leaf caterpillar, *Anomis texana* Riley, is found in all the coastal cotton-producing valleys of Peru, but during ordinary years only in the most northern—and warmer—valleys, Chira and Piura, is *Alabama* found. In these valleys the temperature is sufficiently high and irrigation water is available at such seasons that an almost continuous cotton crop is produced. The effect on the other insect pests of cotton—of which there are many—is an alarming increase in abundance as compared with the southern valleys, yet the continuous supply of fresh cotton leaves throughout the year does not

ensure the continuous presence of *Alabama*. The caterpillars, just as in other countries, first appear in the summer months, which in Peru are January, February and March, strip the plants of their leaves for a season, and as the somewhat cooler months of winter, which in Peru are June, July and August, approach, entirely disappear. In 1928, the caterpillars were last noted in August, and the minimum temperature recorded during the month was 60° F.

A hundred miles or more to the south of Piura is another irrigated valley, where several fields of cotton have been planted during the past year, and although *Anomis* caterpillars occur in these fields, *Alabama* has not been found. The monthly record of temperatures in Lambayeque shows only a few months when the minimum temperatures do not reach 60° F., while at no time during the year did more than a single month pass without a minimum temperature of 65° F. It would appear that somewhere between 60° and 65° F. lies the critical temperature below which *Alabama* caterpillars will not continue to occur in cotton fields.

These observations are based on a single season's experience, yet they are most strikingly confirmed and curiously modified by data supplied by Mr. Gerardo Klinge. (The authenticity of Mr. Klinge's observations may be judged by the fact that it was he who first in Peru noted and pointed out the difference between *Anomis* and *Alabama* caterpillars, obtained determinations from Dr. Dyar, and published the results of his investigations in a local agricultural journal, *La Riqueza Agricola,* in 1912.) The summer temperatures of the year 1912 were abnormally high in Peru, and *Alabama* caterpillars were an unexpected and most serious pest in all the coastal cotton-growing valleys, even to the most southern, yet in the next year, or in succeeding years with normal weather, *Alabama* did not again appear. In 1922 the summer was again abnormally warm, while 1925 was the most exceptional year in a generation, when it rained all

along the coast of Peru, the rainfall being accompanied by high temperatures, and in both of these years *Alabama* appeared in many of the coastal valleys of Peru south of Piura.

The connection between high temperature and the presence of *Alabama* is, indeed, too obvious to be a mere accident. It is true that the caterpillars will continue to eat and transform to pupae and adults even though the nights begin to get cold, but the moths do not oviposit, and no new generation is started. Instead, they appear impelled to flight, undirected and aimless it would seem to us, and probably resulting in the destruction of by far the greater number of moths affected, but also resulting in the continuation of the species by those few individuals which happen to fly towards warmer regions and fresh cotton fields.

We know that the moths found in Canada in the fall have come from the cotton-producing states of the United States, for there is no other plant that the caterpillars will eat but cotton. We know that the moths can fly enormous distances. Is it possible that it is the moths impelled by the oncome of cooler weather south of the equator that are responsible for the first generation of caterpillars that appears in the cotton fields of the West Indies and the southern United States? And that it is the first mildly cool nights in Haiti and Texas that are responsible for starting the reverse flights of moths that reinfest with *Alabama* caterpillars the cotton fields of northern Peru?

If, on the contrary, there is some central, tropical region where the caterpillars find a constant supply of food and are present throughout the year, what is the stimulus that starts the moths on their flights to distant cotton regions, and to the even more distant regions where it is too cold for cotton ever to grow?

SHORTER ARTICLES AND DISCUSSION

SPECIFIC DIFFERENCES BETWEEN THE AMEBAS *MAYORELLA BIGEMMA* AND *M.*(?) *DOFLEINI*

In *Science* for July 27, 1928 (page 84), Johnson states that the description of *dofleini* (Neresheimer, '05) "agrees in all the essential details with the diagnostic characteristics of a rhizopod described by Schaeffer ('18) as a new species under the name . . . *bigemma.*" Mast ('28) expresses a similar view. Johnson further "presumes" that since I did not quote Neresheimer I "merely overlooked his work."

Now since the ameba I described as *bigemma* is a very important experimental animal and since a number of observations on some of its activities have appeared in the literature, it is of course highly desirable that the specific qualities which distinguish it from other species are well understood.

I can not agree with Johnson and Mast that the two descriptions agree in all essential details. I have carefully read the descriptions again and I can not discover that they agree in any essential details nor in any other details. I have been interested for a number of years in specific differences in amebas and in 1920 (p. 38), I stated that "different species are different, even to the smallest detail." I have examined under the microscope over eighty species of live amebas (fresh-water, marine, soil and parasitic), many of them in considerable number and for considerable periods of time, and I have not yet found any "detail" which, when sufficiently carefully measured, proved to be identical in any two species. This is in harmony with observations on specific differences in other animals and in plants and with the results of present-day genetical research.

Adopting this point of view, it will only be necessary to show one or more measurable constant differences between two putative species to establish a specific distinction. For the present purpose it will be sufficient to take the nuclei of the two species *bigemma* and *dofleini,* as described, for the nucleus has long been held as the most reliable morphological criterion for specific differentiation, although as a matter of fact, in some species this is not the case.

In comparing the size of the nuclei Johnson makes a serious mistake in comparing the nuclear sizes of the two species without taking into account the difference in the size of the amebas. For, within ordinary limits of environmental conditions, the diameter of the nucleus varies directly as the length of the ameba in ordinary locomotion. This is based on many hundreds of measurements of *Chaos diffluens, Metachaos discoides, Polychaos dubia, Flabellula citata* and *Rugipes bilzi,* of which the results for the first three species mentioned were published (Schaeffer, '16; '20, p. 38) while sufficient data were given in my 1918 paper to compute the size proportion for *bigemma.* I stated specifically in my description of *bigemma* (p. 8) that a *bigemma* of 200 μ length has a nucleus 12 μ in diameter, with a central nuclear chromatin mass (karyosome) about 6.5 μ in diameter. Another culture of large amebas (500 μ) had nuclei 28 μ in diameter and karyosomes 14 μ in diameter. Nuclear size and body size thus vary together just as they have been found to vary in other cells. And the ratios in amebas also, so far as definitely known, are specifically constant.

Now on measuring several of Neresheimer's figures (G, a, p. 151; M, p. 159; 2, 16, Taf. 7) which were drawn to scale, it is found that an ameba of 200 μ would have a nucleus of about 33 μ diameter and a karyosome of about 19 μ. This ratio also holds for Neresheimer's statement that amebas of 80 μ to 150 μ length (average size, say, of 120 μ) have nuclei about 20 μ in diameter.

RATIO OF LENGTH OF AMEBA TO DIAMETER OF NUCLEUS

	length	diam. of nucleus	diam. of karyosome
dofleini	200 μ	33 μ	19 μ
bigemma	200	12	6.5

A *dofleini* of 200 μ length therefore has a nucleus *twenty times as large in volume* as that of a *bigemma* of the same size, and according to the descriptions these are *constant specific differences.* No such range in nuclear size, nor anything approaching it, is known to me to exist in any normal culture of any one species of ameba. This great and constant difference would be sufficient in itself to set these two species apart taxonomically, but there are a number of others equally striking. Thus the periphery of the karyosome of *bigemma* is made up of permanent discrete grains of nearly uniform size, whereas that of

dofleini presents a fine alveolar or honeycomb (feinwabige) structure (p. 148, 151; fig. 1, 2, Taf. 7). The microscopic equipment Neresheimer used would have enabled him easily to distinguish between a granular and an alveolar structure. Moreover the alveoli Neresheimer describes are on the surface of the karyosome and the little round bodies of which he speaks float in the nuclear sap between the karyosome and the nuclear membrane. Then, again, the nuclear membrane in *dofleini* is often strikingly deformed by infoldings like that of old and large individuals of *Chaos diffluens.* Nothing like it is seen in *bigemma.* A similar specific difference in nuclear membrane folds is also constant between *Metachaos discoides,* where it has never been seen to occur, and *Chaos diffluens* where, as was just said, it often occurs in old and large individuals (Schaeffer, '16; '18; '20, p. 38). This specific difference might be due in *Chaos diffluens* to the larger size of the nucleus, although it is not known that this is the case. But a difference in size can not be assumed to cause the infoldings in *dofleini* as compared with *bigemma,* since the nuclei in the larger *bigemma* culture were much larger, absolutely, than those of average sized *dofleini.*

The peripheral grains of the karyosome, which Johnson thinks are similar in the two species, are in reality also quite different things. In *bigemma* they are of very nearly uniform size, more deeply and distinctly colored bluish green, in the living nucleus, than any other part of the nucleus. In *dofleini* these round bodies may be absent (fig. F; G, a, p. 151) or there may be two or three floating in the nuclear sap (fig. 2, 16, Taf. 7) or there may be scores which fill nearly all the space within the nuclear membrane in markedly asymmetrical arrangement (fig. 12, 13, Taf. 7). Moreover, the *dofleini* grains are *clear* (heller und hyaliner . . . als das Karyosom selbst) and of very uneven size (fig. 12, Taf. 7) and often coalesce with each other and "öfters kurz nach ihrer Entstehung (extrusion from the karyosome) durch die Kernmembran ins Plasma hinausgestossen." In short, these bodies in the *dofleini* nucleus are very extraordinary in their origin and behavior and, so far as I know, unique among amebas. They are entirely different structures in every particular from the karyosome grains of *bigemma.* And further, the occurrence of grains such as *dofleini* possesses is not recorded

for *bigemma*. These differences are clearly indicated in the descriptions as well as in the figures.

This thoroughgoing difference between the nuclei of *bigemma* and *dofleini* extends to the rest of these amebas, of which, however, only two features need be discussed insofar as this taxonomic discussion is concerned. The first of these has to do with the crystals. In *bigemma* these are twin structures, of hour-glass shape in the majority of cases and attached to small spheres at the middle of the crystals. In *dofleini* the crystals are "meist ziemlich regelmässig *stabförmig* . . . unregelmässige Formen kommen nicht selten vor, so keulenförmig verdickte, *selten auch an beiden Enden verdickte* (*Hantelform*)" (pp. 157). The *dofleini* crystals, while attached to spheres, are not permanently attached by their middle points, but the crystals slide around on the spheres frequently with the spheres attached to one of the ends of the crystals. This sliding around over the spheres by the crystals has not been described for *bigemma*. These structures also differ in the two species in a thoroughgoing manner, as far as one may be safe in matching contentions in literature, and my statement that "*such* crystals attached to spheres in an ameba may be regarded as definitely proving its (*bigemma*) specific identity" expresses a fact of practical taxonomic value.

The second feature to be mentioned has to do with the general form of the ameba and its movements. The pseudopods of *bigemma* are "tapering, tongue-like, more or less conical, usually short, sometimes very long." The shape of the body in locomotion is "most frequently triangular." But Neresheimer says (p. 147) of *dofleini* that "in ihrem Habitus gleicht die Amöba ziemlich der Amoeba verrucosa" but that it is without the characteristic ectoplasmic folds of this species and that it moves more actively. And he makes the important statement, mistranslated by Johnson, that *dofleini* "bewegt sich . . . vermittels breiter, kurzer, kaum vom Körper abgesetzter bruchsackartiger Pseudopodien" (bruchsackartiger = "broken sack-like"—Johnson). Now although it seems very unlikely that any one who has seen a *bigemma* would see general resemblance in it to *verrucosa* and that its pseudopods were "hernia-like, broad and short and barely projecting from the body," this comparison is of comparatively small value since Neresheimer drew too few changes of body shapes and made too few measurements of the points involved.

The status of Doflein's "*vespertilio*" does not bear directly on this case of alleged synonymy and will therefore not be discussed in this brief note.

Johnson is also seriously in error when he concludes that if *bigemma* is a synonym of *dofleini*, then the International Rules or amendments would sanction *Amoeba dofleini* as the correct name. This would be true only of *dofleini*. That the rules do not sanction the name *Amoeba* for a genus of amebas has been brought out by a number of authors, *e.g.*, Leidy, '79; Stiles, '05, '23; Schaeffer, '26. And even if the name *Amoeba* were rendered valid by special vote of the International Commission in the case of *Chaos diffluens* (see Schaeffer, '26), which could be upheld only by having more regard for a system of nomenclature than for scientific considerations, the genus name *Amoeba* would apply to *dofleini* only if one were ready to unite all species of amebas excepting *Chaos chaos* under one genus. If the rules really sanctioned such action, which would amount to prevention of splitting up a genus on scientific grounds, no taxonomist would follow them. Needless to say, the rules are not intended to interfere with the scientific classification of organisms but to aid it.

Insofar as Johnson's argument may have been inspired by my failure to quote Neresheimer, I may say that I did not overlook his paper but read it carefully and had photostats made of several sections, which are still in my files, since I was at the time still carrying on at the University of Tennessee, where the original literature was not available. Failure to quote it simply meant that Neresheimer's *dofleini*, as well as a number of other species, was not sufficiently closely related to the ameba I was describing to warrant its consideration. The foregoing discussion should make this point clear.

That it is of fundamental importance to distinguish definitely between species of organisms is again illustrated in this case, for Mast ('28) records some experimental tests on "*bigemma*," and in the same paper in which he records his results he expresses the opinion that *dofleini* and *bigemma* are the same species. Since he does not give any data on the actual appearance of the ameba he used, it is not possible to tell to which of the two species his statements apply and his work can not therefore be checked, unless *dofleini*, which has been seen only once,

can be found again. For it has already been abundantly shown that two species may resemble each other very closely in a general way and yet be strikingly different in some particulars, as for example, *Trichamoeba sphaerarum* and *T. gumia,* which vary enormously in their reactions to diluted sea-water although in general they resemble each other very closely. Still more closely similar in a general way are *Mayorella bigemma* and the marine *M. conipes,* yet they differ strikingly in a number of so-called details which are of great physiological interest. So, to say that two species of amebas are "essentially similar" is most likely to mean that the writer is inviting critical attention away from these species.

But even though we have what seems to be as adequate a basis for accurately distinguishing between species of amebas as we have in any other group, I, for my part, have not been content to let the matter rest there. Miss Lucy Heathman therefore undertook, partly at my suggestion, to carry through an extended series of serological tests during the past two years of six species of amebas—to date all the species which we have been able to raise in sufficient quantity—to test out further still specific distinctions and relationships. A paper recording these results is ready for the press. This work confirms in a remarkable manner the conception of thoroughgoing specific distinctions and that of the practicability of basing specific, generic and family distinctions on morphology in amebas. This is a great step in advance, since it is a thoroughly tested quantitative method and it should therefore help to remove from rigorous scientific consideration the matching of "contentions" in literature regarding specific (and other!) differences, which after all is a form of indoor sport that might well be generously reserved for those who can no longer get after the bugs.

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BIRDS AS HOSTS FOR THE COMMON CHIGGER

DURING the month of July, 1928, the writer, in cooperation with Mr. C. S. East, of the U. S. National Museum, made a survey of the ectoparasites of many birds, small mammals, reptiles and amphibians occurring in southeastern Virginia and northeastern North Carolina. Collections were made at Virginia Beach, Euclid, Thalia, Wallaceton and Lake Drummond in Virginia and on Roanoke Island in North Carolina. The chief object of this survey was to increase our knowledge in regard to the natural hosts of chiggers.

In all, eighty-three birds were taken, representing thirty-four species. Most of these were small land birds. To our surprise many of the birds were found to be infested with the common North American chigger, *Trombicula irritans* (Riley). Our common chigger had previously been reported from prairie chickens and quails and had been known to be a serious pest of chickens, but that it occurred on birds generally was hardly to be expected. The birds examined, together with the number of each kind taken, were as follows:

Little green heron, *Butorides virescens virescens*	1
Downy woodpecker, *Dryobates pubescens*	1
Red-headed woodpecker, *Melanerpes erythrocephalus*	1
Chimney swift, *Chaetura pelagica*	1
Wood pewee, *Myiochanes virens*	1
Acadian flycatcher, *Empidonax virescens*	1
Flycatcher (species?)	1
Orchard oriole, *Icterus spurius*	6
English sparrow, *Passer domesticus domesticus*	1
Henslow's sparrow, *Passerherbulus henslowi henslowi*	1
Chipping sparrow, *Spizella passerina passerina*	2
Field sparrow, *Spizella pusilla pusilla*	7
Towhee, *Pipilo erythrophthalmus erythrophthalmus*	5

Indigo bunting, *Passerina cyanea*	2
Purple martin, *Progne subis subis*	2
Tree swallow, *Iridoprocne bicolor*	1
White-eyed vireo, *Vireo griseus griseus*	2
Black and white warbler, *Mniotilta varia*	1
Prothonotary warbler, *Protonotaria citrea*	13
Yellow-throated warbler, *Dendroica dominica dominica*	1
Pine warbler, *Dendroica vigorsi vigorsi*	5
Prairie warbler, *Dendroica discolor*	1
Kentucky warbler, *Oporornis formosus*	1
Maryland yellowthroat, *Geothlypis trichas trichas*	6
Hooded warbler, *Wilsonia citrina*	4
Redstart, *Setophaga ruticilla*	2
Warbler (species?)	1
Catbird, *Dumetella carolinensis*	3
Brown thrasher, *Toxostoma rufum*	1
Carolina wren, *Thryothorus ludovicianus ludovicianus*	4
Long-billed marsh wren, *Telmatodytes palustris palustris*	2
Blue-gray gnatcatcher, *Polioptila caerulea caerulea*	1
Bluebird, *Sialis sialis sialis*	1

Of the thirty-four species taken seven were found to act as chigger hosts. Probably if a larger number of specimens of each species had been examined this number would have been more than doubled. The birds found infested with chiggers were:

Field sparrow
Towhee
Prothonotary warbler
Maryland yellowthroat
Redstart
Carolina wren
Blue-gray gnatcatcher

All the four specimens of the Carolina wren taken were found to be heavily infested with chiggers. On the other hand, of the thirteen specimens of the prothonotary warbler taken only one was found to be infested. It had just four chiggers, all attached at the base of a pin feather on the breast. While the Carolina wren was found to be heavily infested, the long-billed marsh wren, on the other hand, had no chiggers. This is easily explained on the basis of the habits of the marsh wren, which keep it in situations that are flooded with water much of the time, hence are free from chiggers.

It is not known as yet to what degree birds enter into our present picture of the natural host relationships of chiggers.

It would appear, however, that in many localities ground-frequenting birds are of more importance as hosts for chiggers than either the rabbit or box turtle. The rabbit, of all of our wild mammals, and the box turtle, of all our reptiles, appear to be important hosts of chiggers. This is more particularly true because of the wide distribution and great abundance of these two hosts.

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